Figure 1A

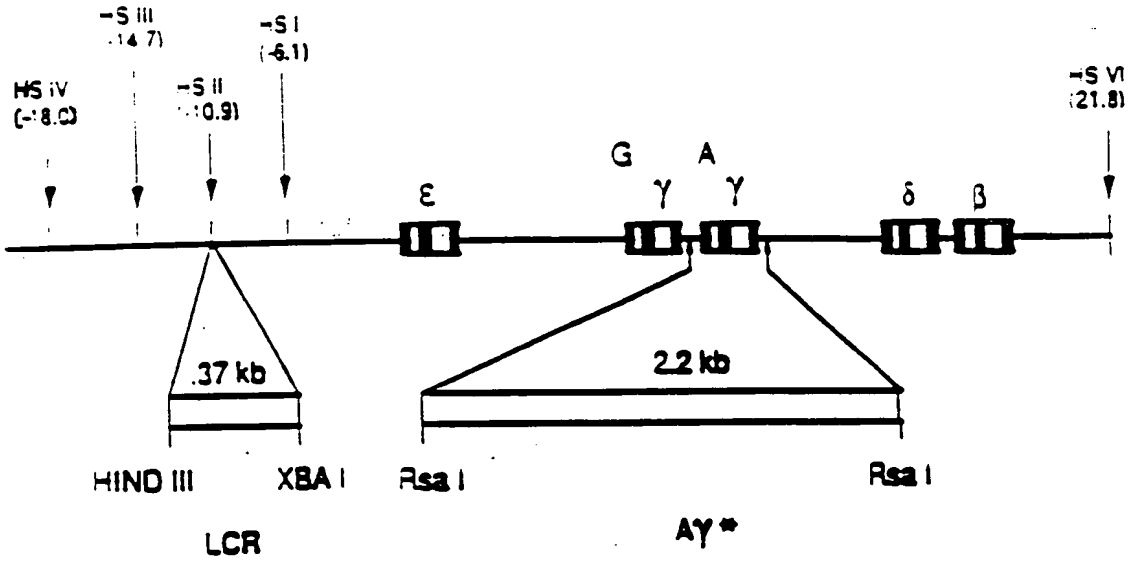


Figure 1B

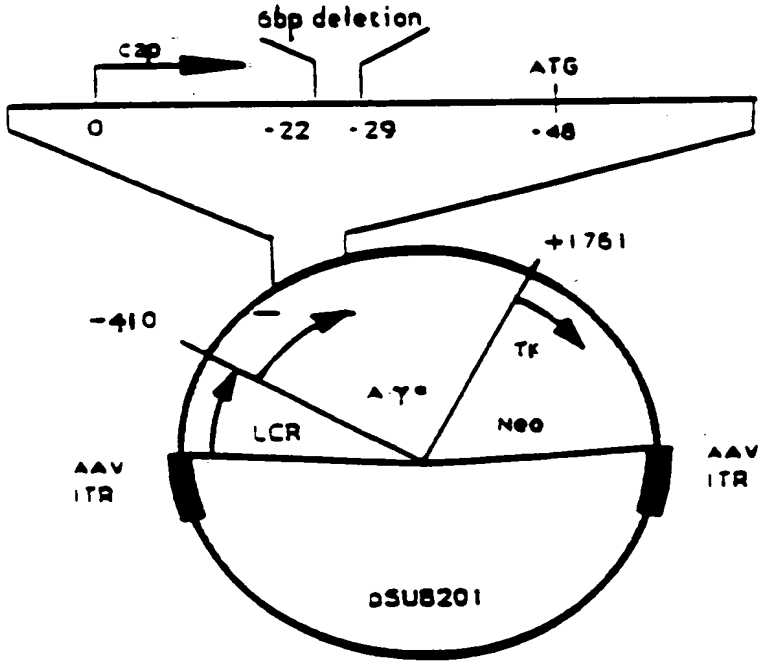


Figure 2A (+HS2) POOLS

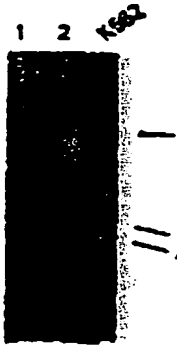


Figure 2B (+HS2) CLONES

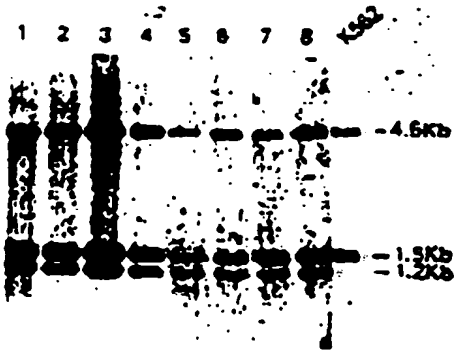


Figure 2C (-HS2)

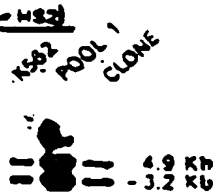


FIG 2D

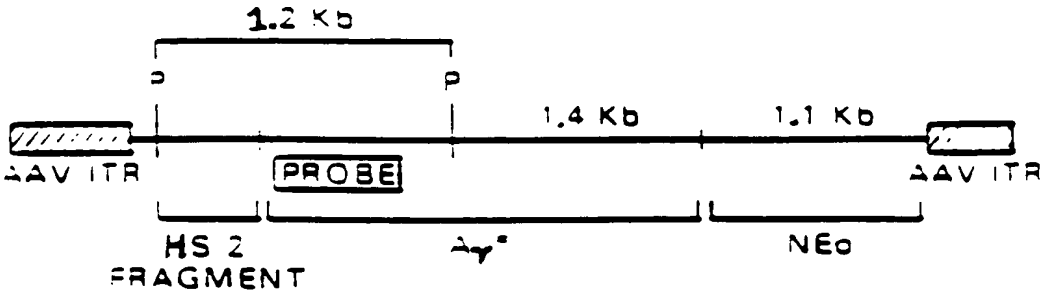


FIG. 3

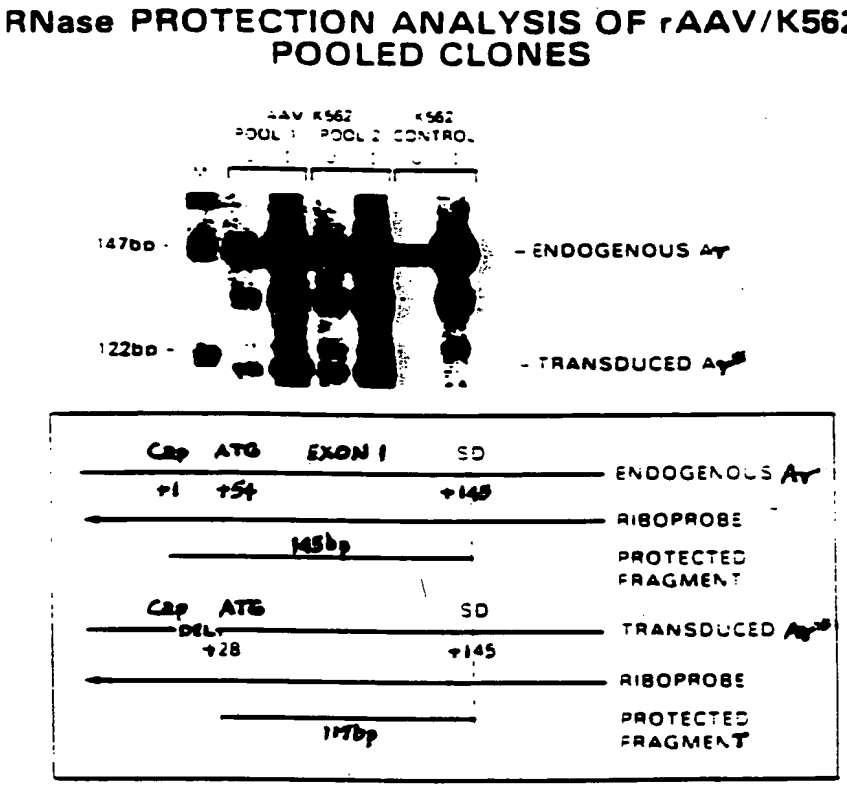

RNase PROTECTION ANALYSIS OF rAAV/K562 POOLED CLONES
AAV K562 POOL 1
AAV K562 POOL 2
K562 CONTROL
1470b
1220b
- ENDOGENOUS Ap
- TRANSDUCED Ap
Cap
ATG
EXON 1
SD
+1
+54
+145
ENDOGENOUS Ap
RIBOPROBE
145bp
PROTECTED FRAGMENT
Cap
ATG
DEL
+28
SD
+145
TRANSDUCED Ap
RIBOPROBE
117bp
PROTECTED FRAGMENT

FIG. 4

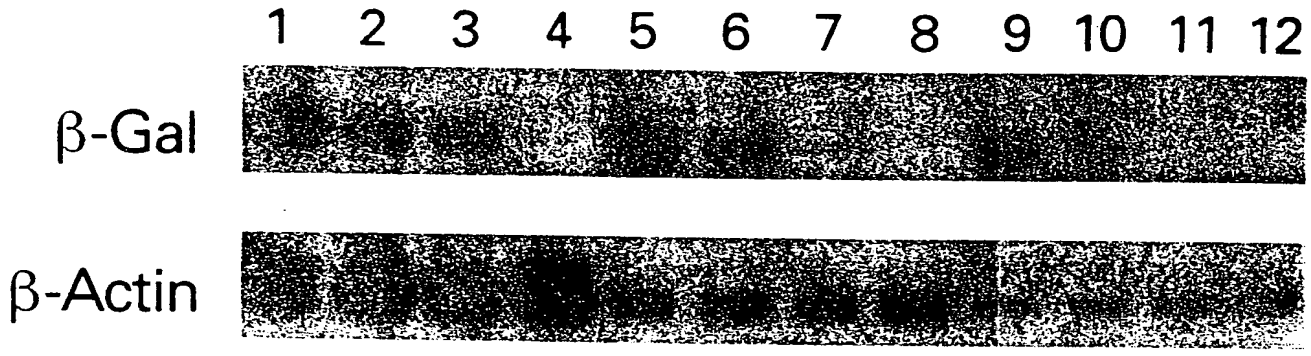


FIG. 5

FIG. 6

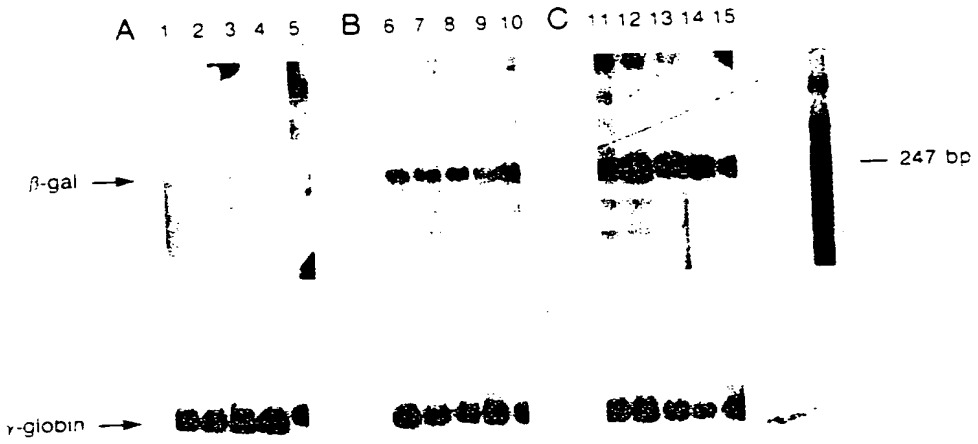


FIG. 7

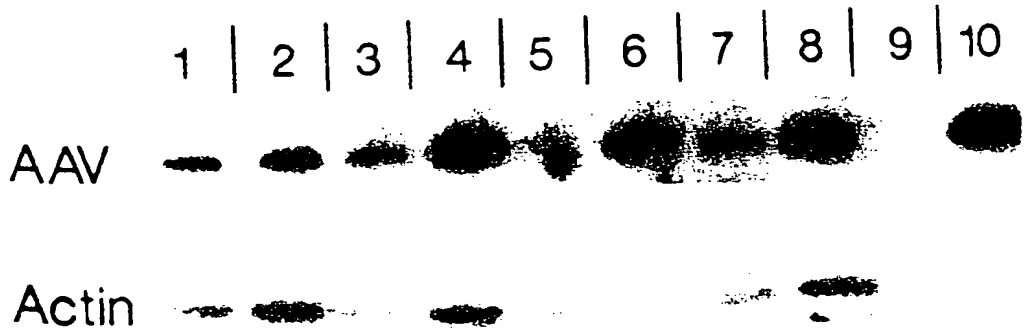
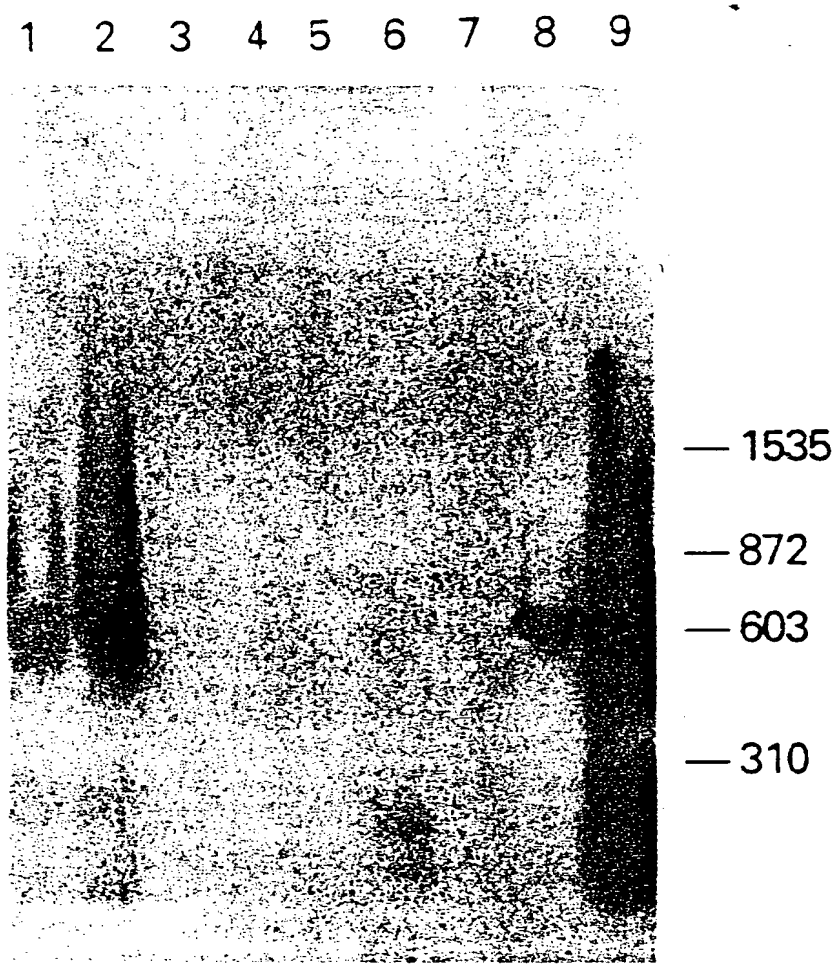


FIG. 8


1 2 3 4 5 6 7 8 9
1535
872
603
310

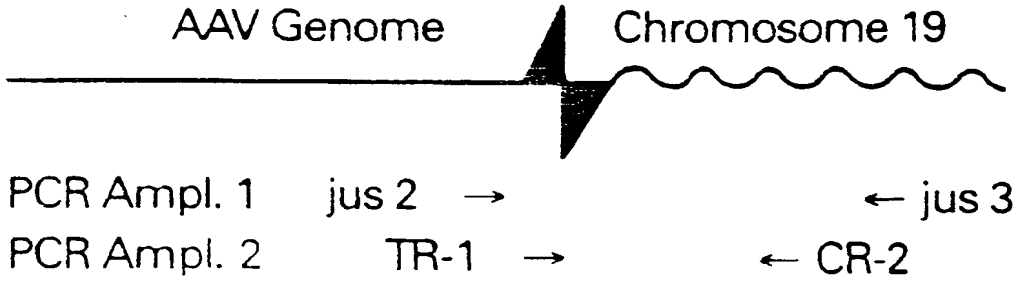

AAV Genome
Chromosome 19
PCR Ampl. 1
jus 2 →
← jus 3
PCR Ampl. 2
TR-1 →
← CR-2

FIG. 9

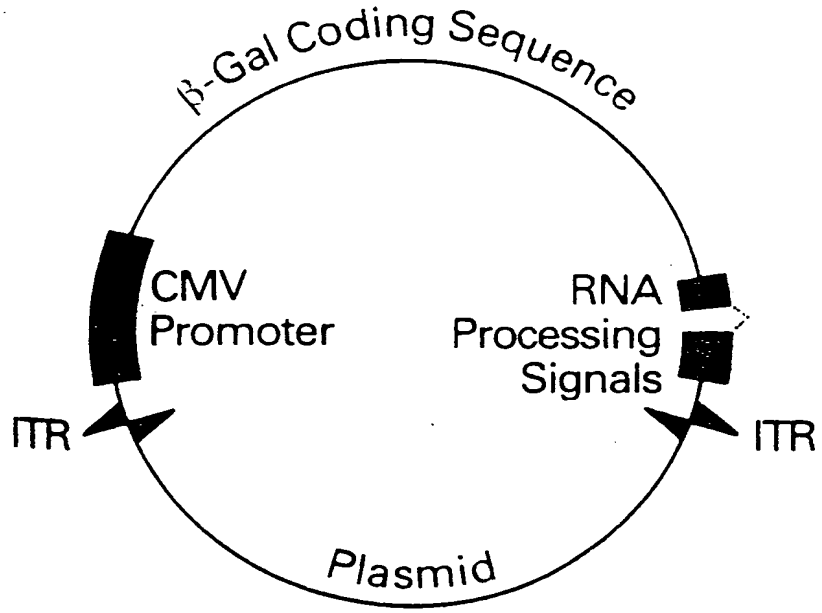

β-Gal Coding Sequence
CMV
Promoter
RNA
Processing
Signals
ITR
ITR
Plasmid

FIG. 10

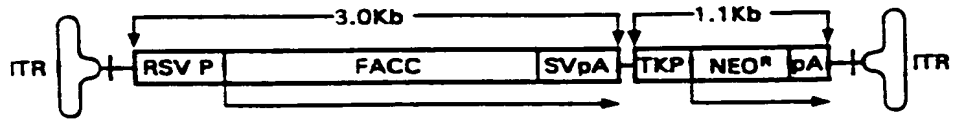


FIG. 11

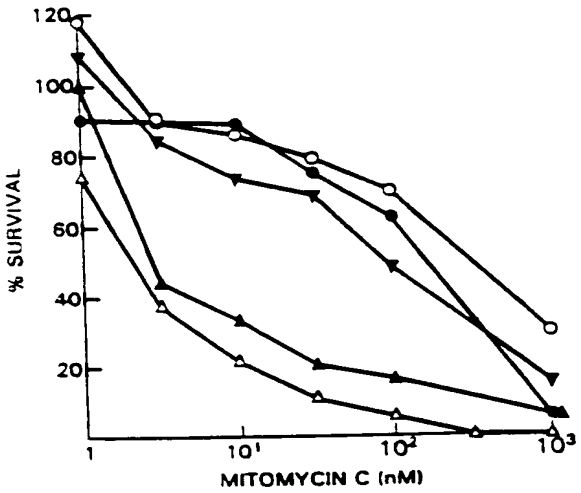


FIG. 12

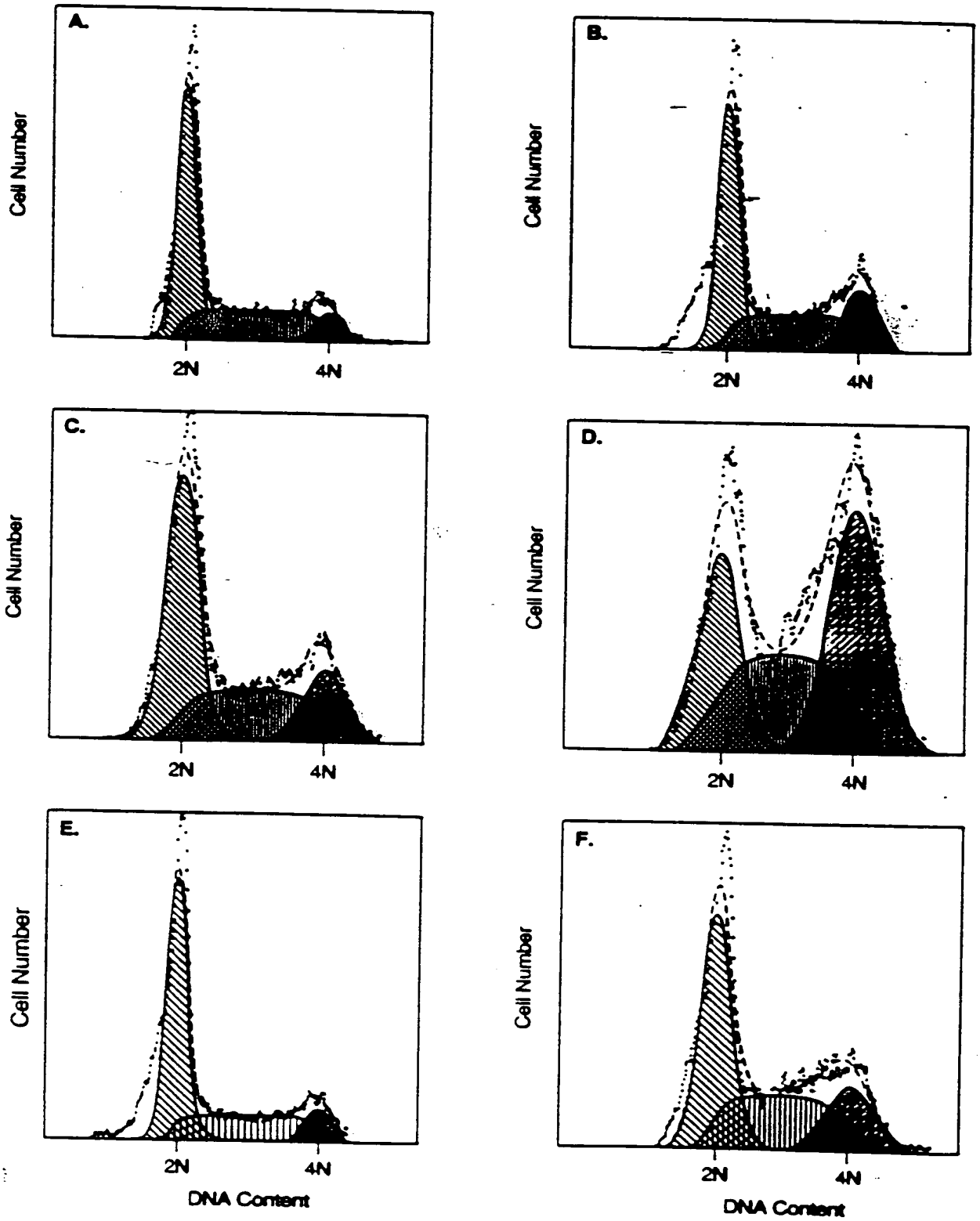
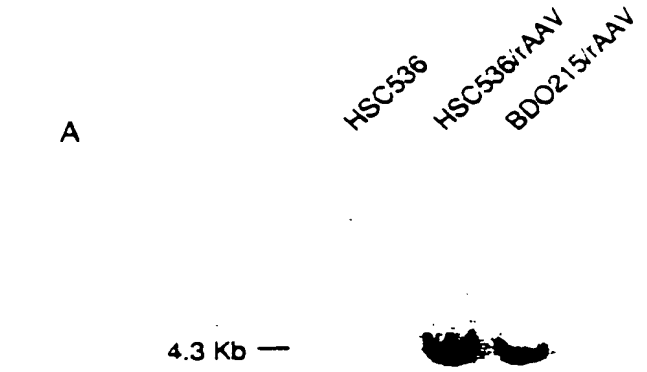


FIG. 13


A
HSC536
HSC536/rAAV
BDO215/rAAV
4.3 Kb

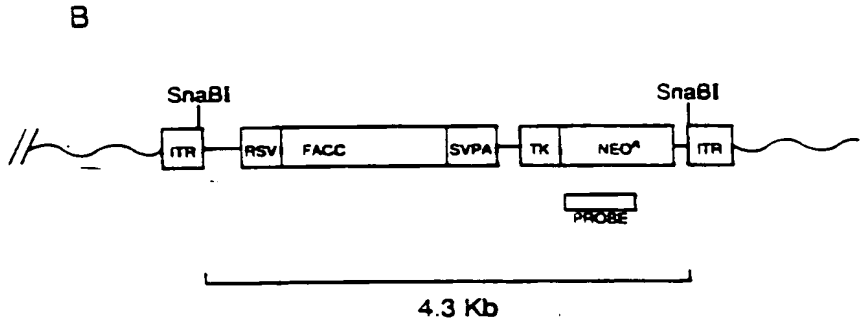

B
SnaBI
SnaBI
ITR
RSV
FACC
SVPA
TK
NEO^R
ITR
PROBE
4.3 Kb

FIG. 14

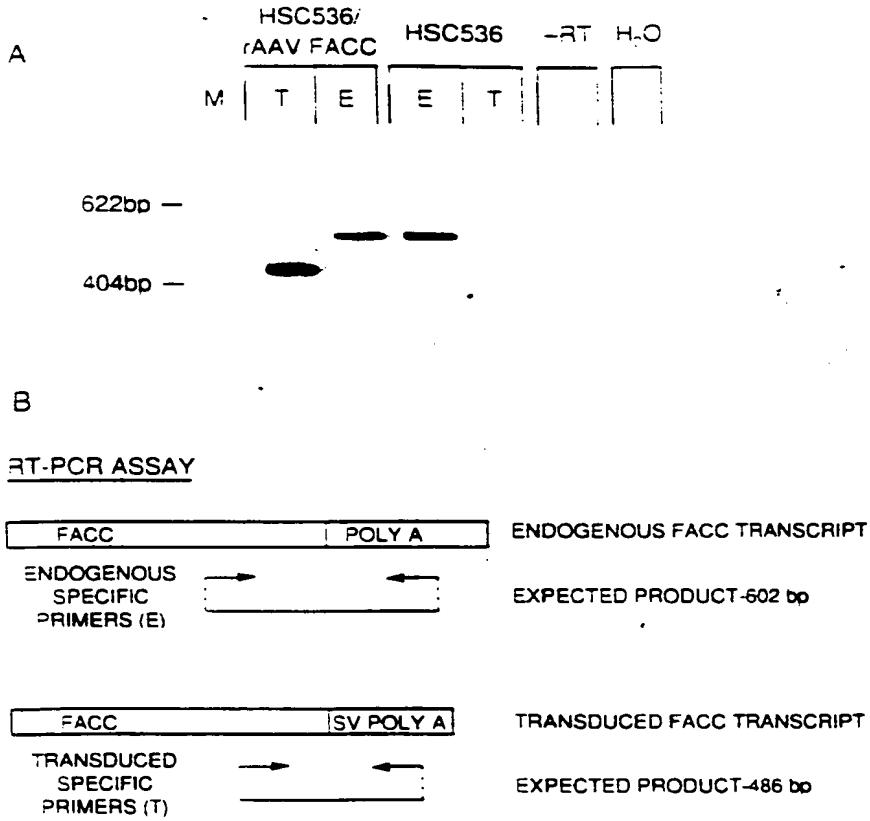

A
HSC536/
rAAV FACC
HSC536
-RT
H2O
M
T
E
E
T
622bp
404bp
B
RT-PCR ASSAY
FACC
POLY A
ENDOGENOUS FACC TRANSCRIPT
ENDOGENOUS SPECIFIC PRIMERS (E)
EXPECTED PRODUCT-602 bp
FACC
SV POLY A
TRANSDUCED FACC TRANSCRIPT
TRANSDUCED SPECIFIC PRIMERS (T)
EXPECTED PRODUCT-486 bp

FIG. 15

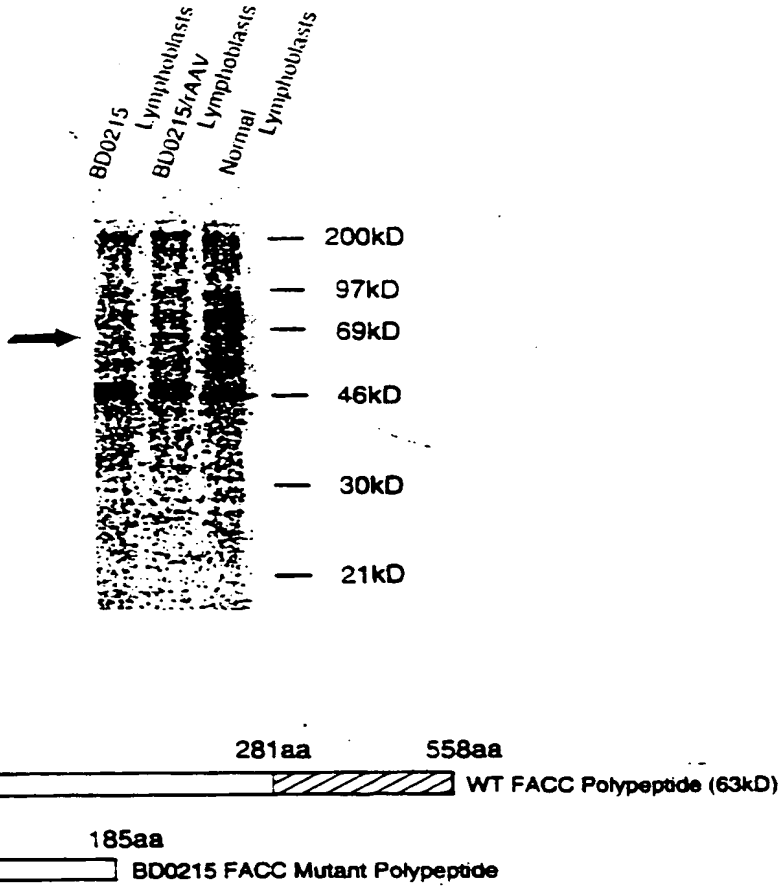

BD0215 Lymphoblasts
BD0215/rAAV Lymphoblasts
Normal Lymphoblasts
200kD
97kD
69kD
46kD
30kD
21kD
281aa
558aa
WT FACC Polypeptide (63kD)
185aa
BD0215 FACC Mutant Polypeptide

FIG. 16

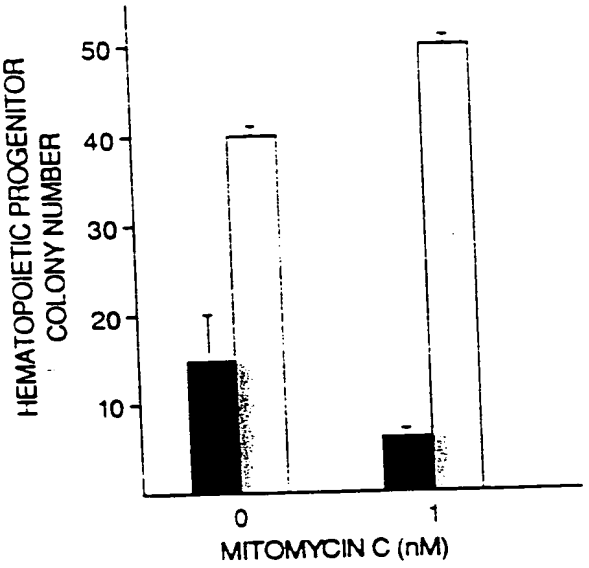

HEMATOPOIETIC PROGENITOR
COLONY NUMBER
50
40
30
20
10
0
1
MITOMYCIN C (nM)

FIG. 17

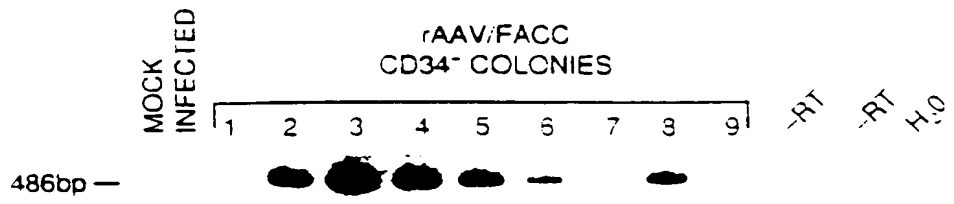

MOCK INFECTED
rAAV/FACC
CD34⁻ COLONIES
1
2
3
4
5
6
7
8
9
-RT
-RT
H2O
486bp

FIG. 18

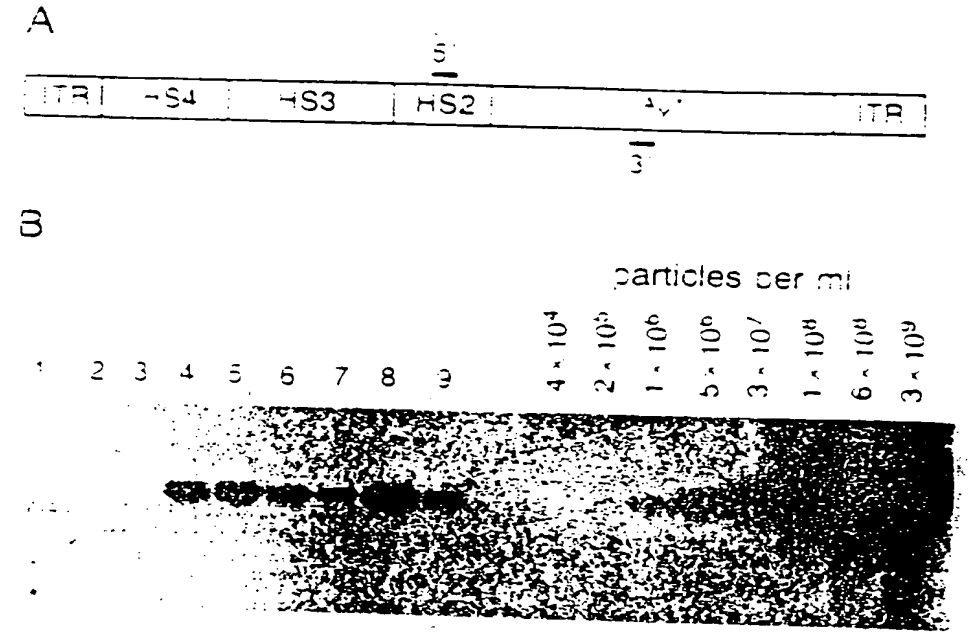

A
5'
ITR
HS4
HS3
HS2
ITR
3'
B
particles per ml
1 2 3 4 5 6 7 8 9
4 × 10⁴
2 × 10⁵
1 × 10⁶
5 × 10⁶
3 × 10⁷
1 × 10⁸
6 × 10⁸
3 × 10⁹
C
d
m

FIG. 19

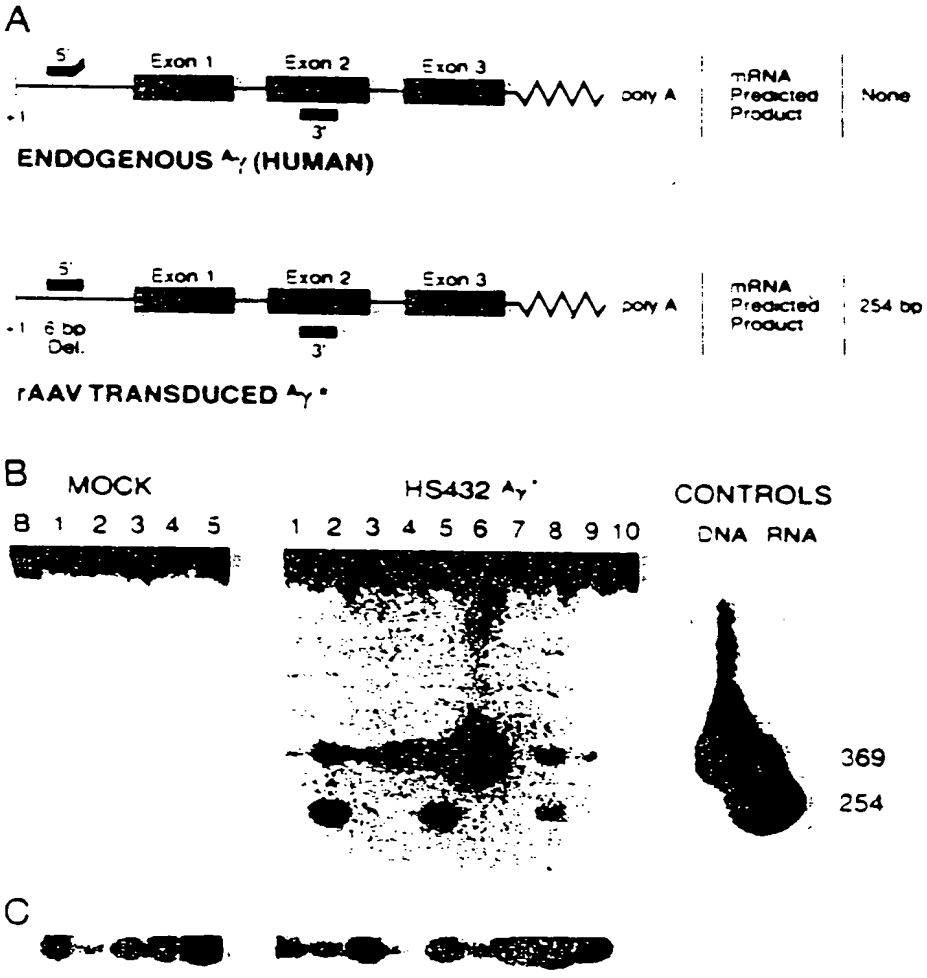

A
5'
Exon 1
Exon 2
Exon 3
poly A
+1
3'
mRNA Predicted Product
None
ENDOGENOUS Aγ (HUMAN)
5'
Exon 1
Exon 2
Exon 3
poly A
+1 6 bp Del.
3'
mRNA Predicted Product
254 bp
rAAV TRANSDUCED Aγ*
B
MOCK
B 1 2 3 4 5
HS432 Aγ*
1 2 3 4 5 6 7 8 9 10
CONTROLS
DNA RNA
369
254
C

FIG. 20

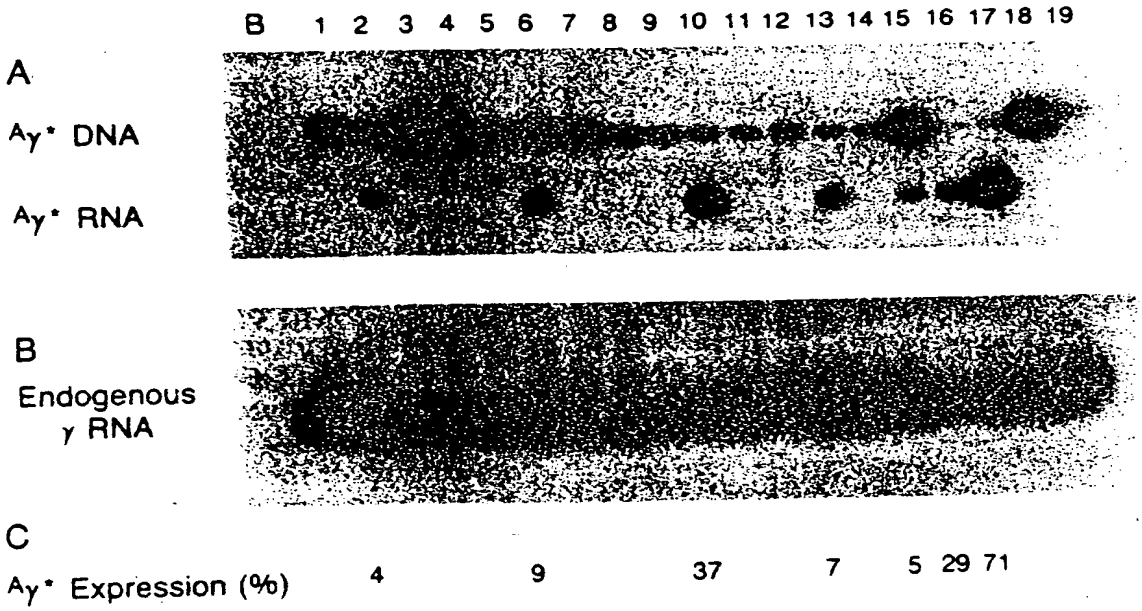

B 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
A
Aγ* DNA
Aγ* RNA
B
Endogenous γ RNA
C
Aγ* Expression (%)
4
9
37
7
5
29
71

FIG. 21

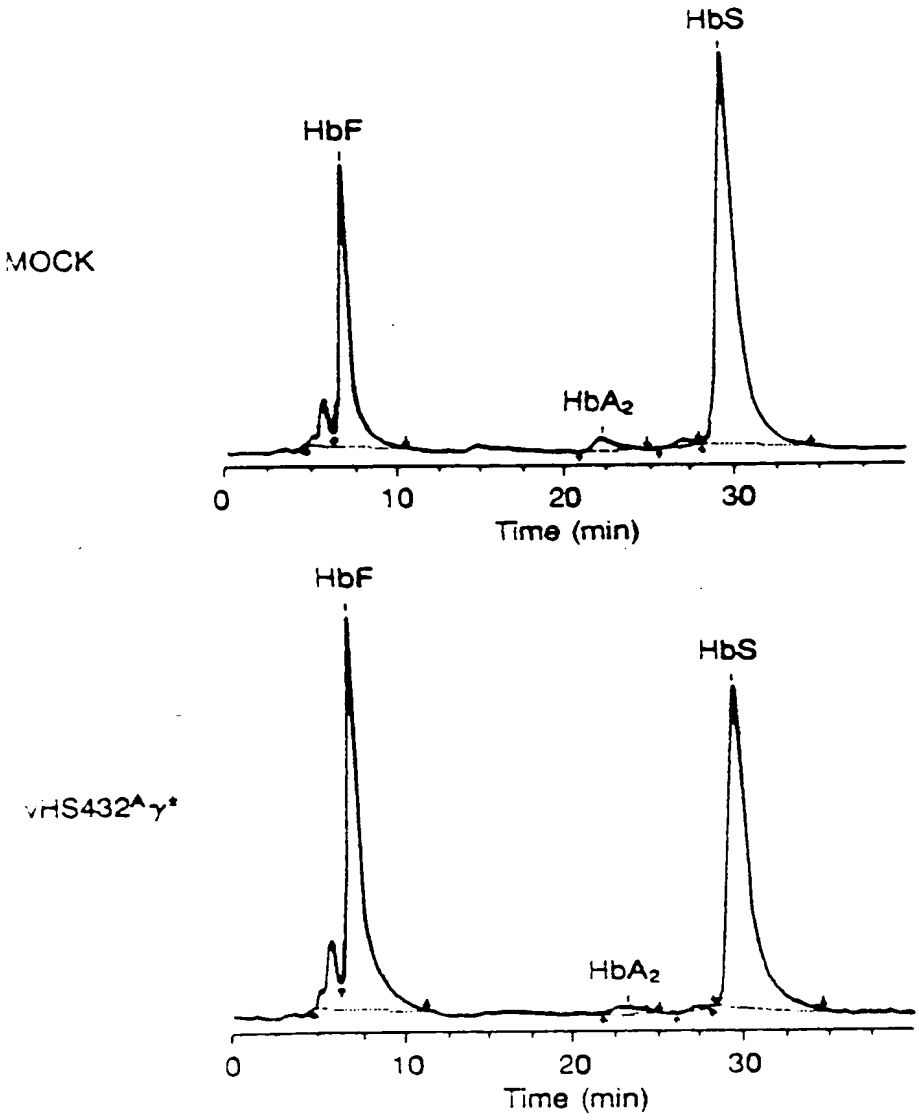

MOCK
HbF
HbS
HbA2
0
10
20
30
Time (min)
HbF
HbS
HbA2
0
10
20
30
Time (min)

Figure 1A

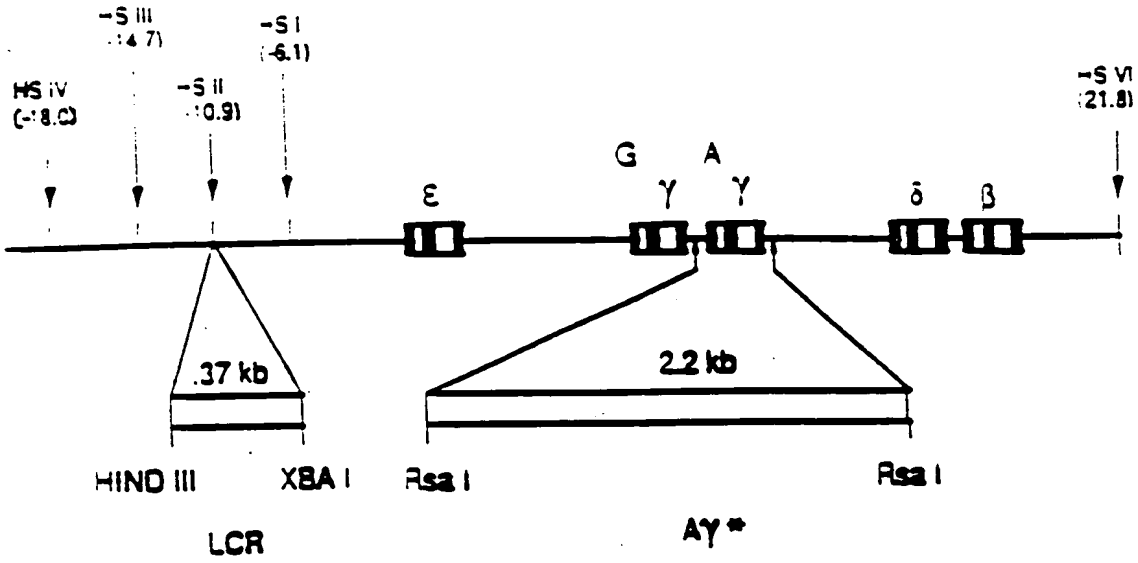


Figure 1B

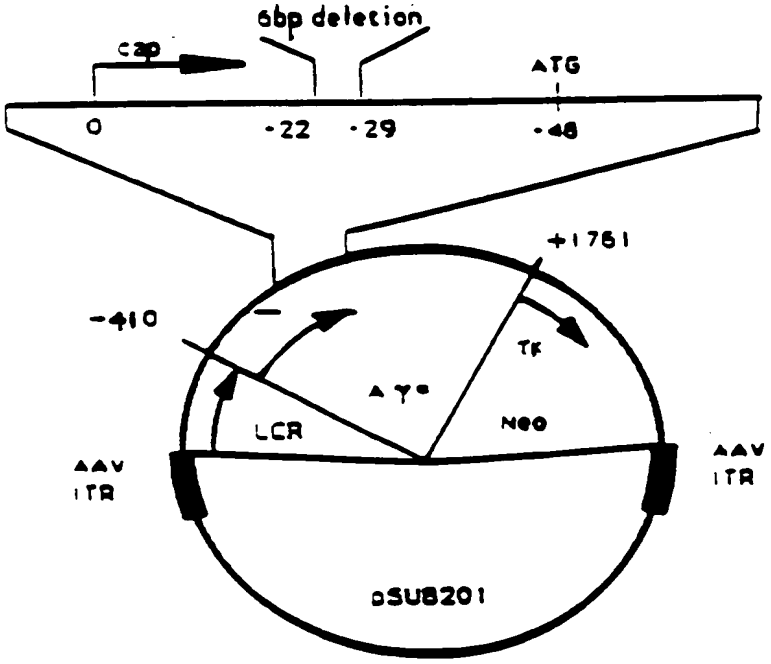


Figure 2A (+ HS2) POOLS

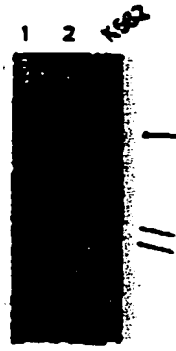


Figure 2B (+ HS2) CLONES

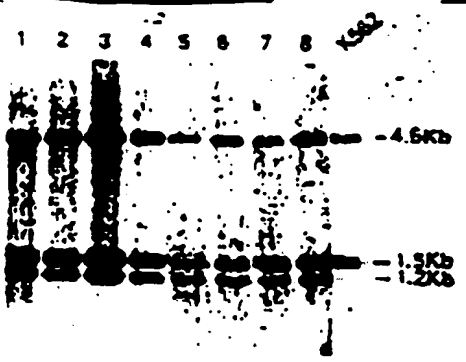


Figure 2C (- HS2)

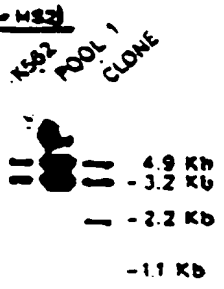


FIG 2D

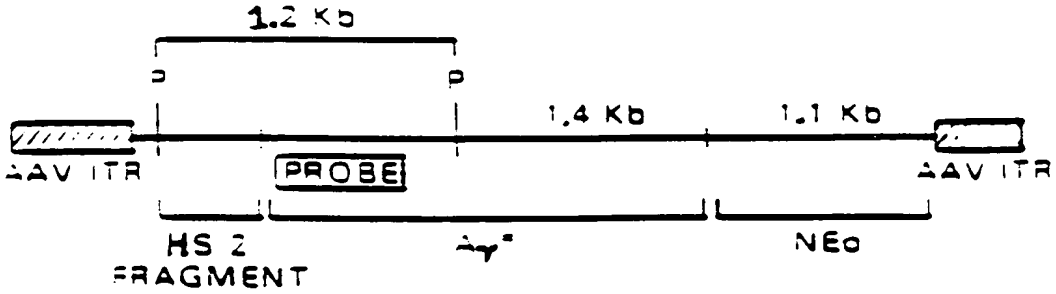


FIG. 3

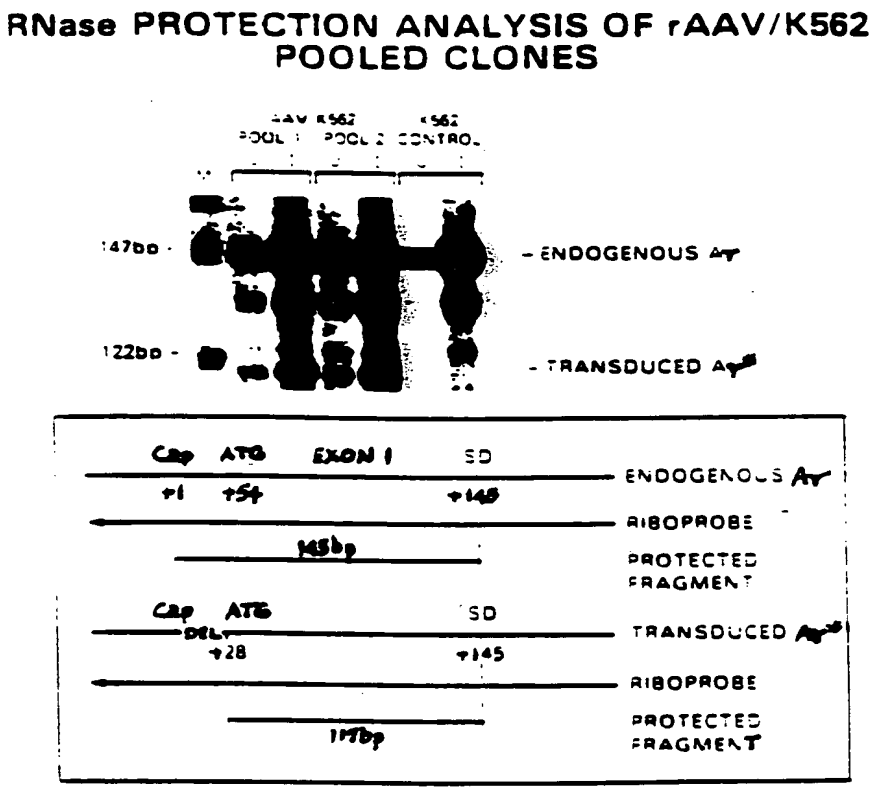

RNase PROTECTION ANALYSIS OF rAAV/K562 POOLED CLONES
AAV K562 POOL 1
K562 POOL 2
K562 CONTROL
147bp
122bp
ENDOGENOUS AT
TRANSDUCED AT
Cap
ATG
EXON 1
SD
+1
+54
+145
ENDOGENOUS AT
RIBOPROBE
145bp
PROTECTED FRAGMENT
Cap
ATG
DEL
SD
+28
+145
TRANSDUCED AT
RIBOPROBE
117bp
PROTECTED FRAGMENT

FIG. 4

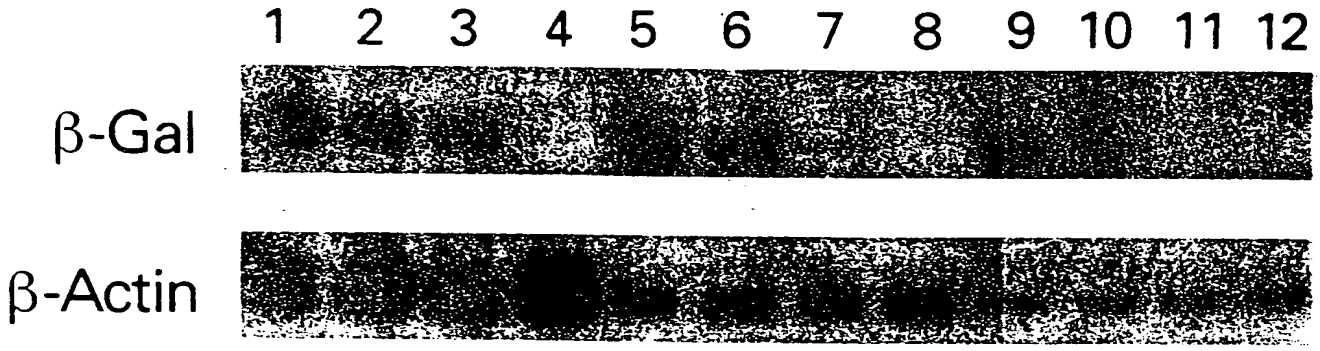


FIG. 5

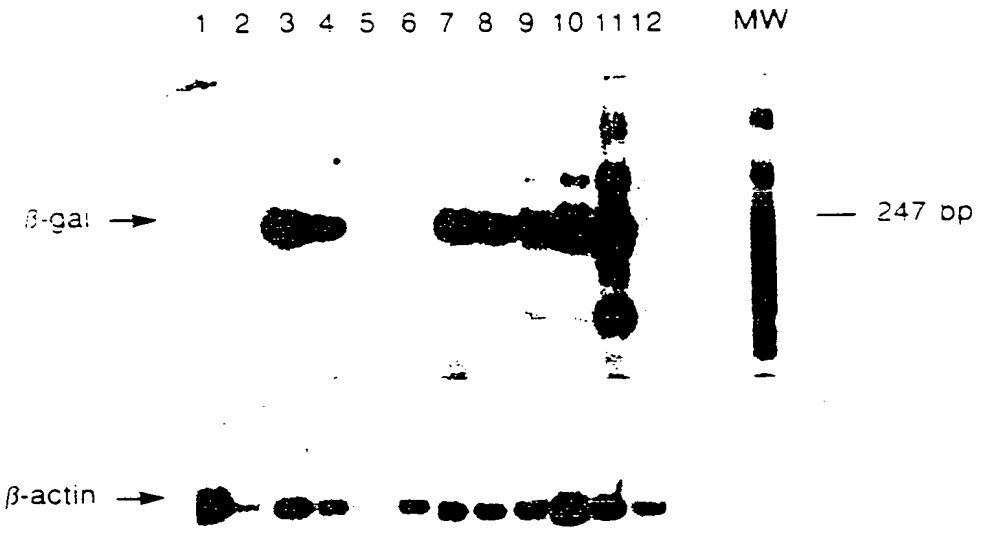


FIG. 6

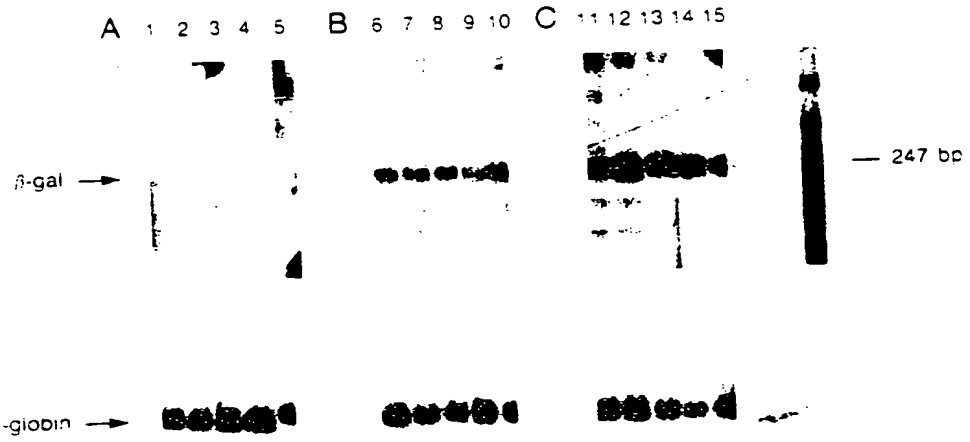


FIG. 7

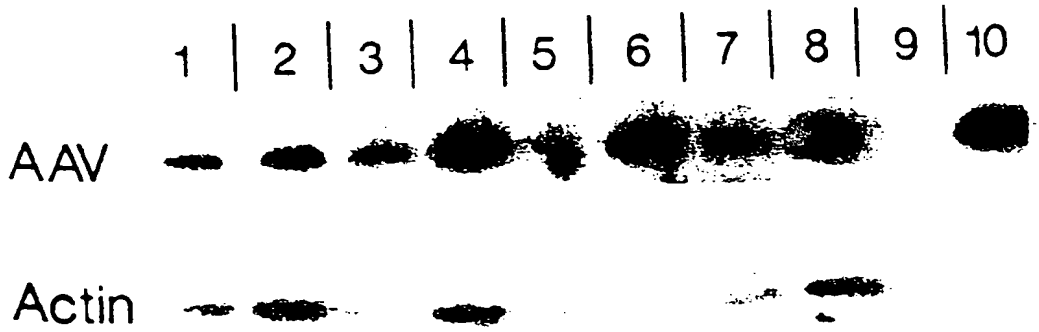
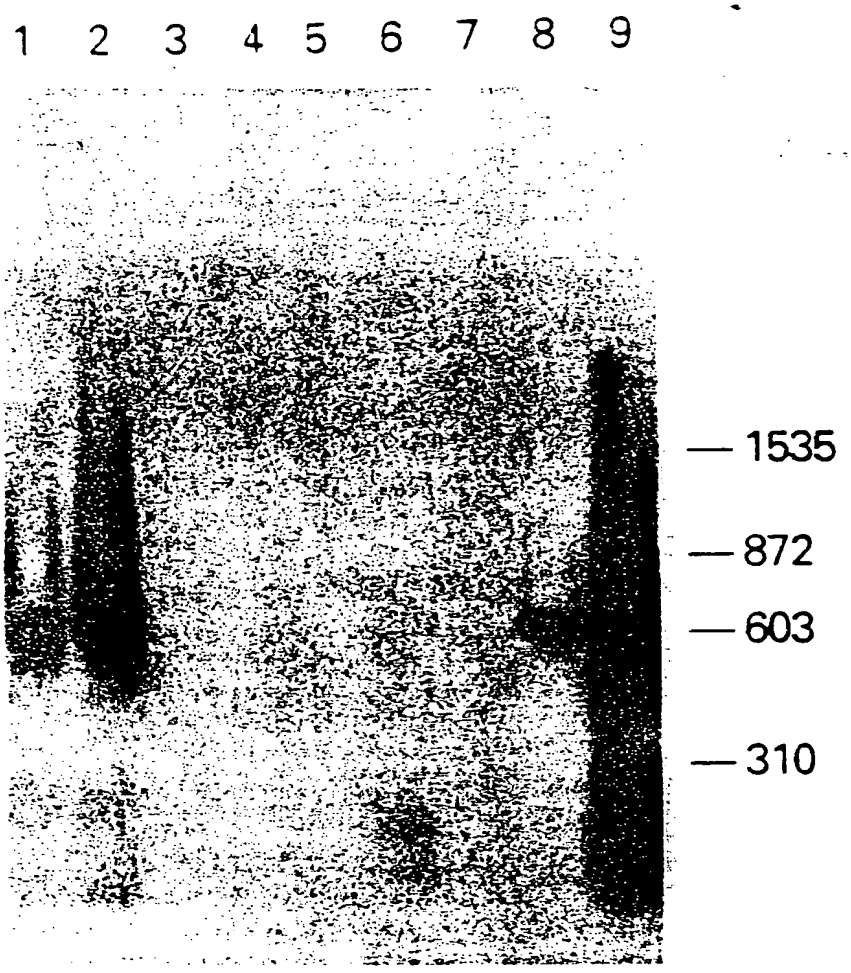


FIG. 8


1 2 3 4 5 6 7 8 9
1535
872
603
310

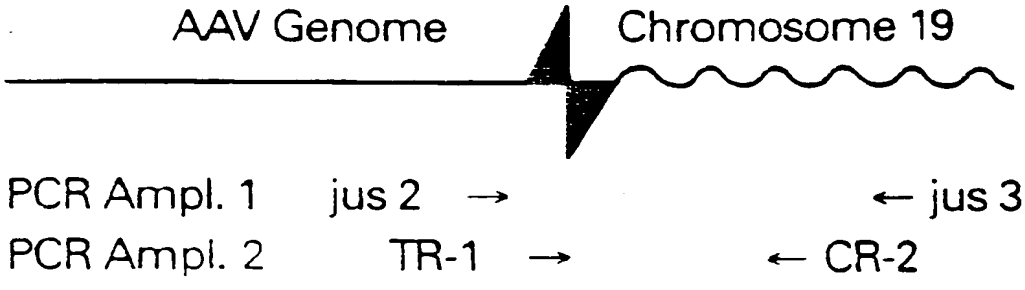

AAV Genome
Chromosome 19
PCR Ampl. 1
jus 2 →
← jus 3
PCR Ampl. 2
TR-1 →
← CR-2

FIG. 9

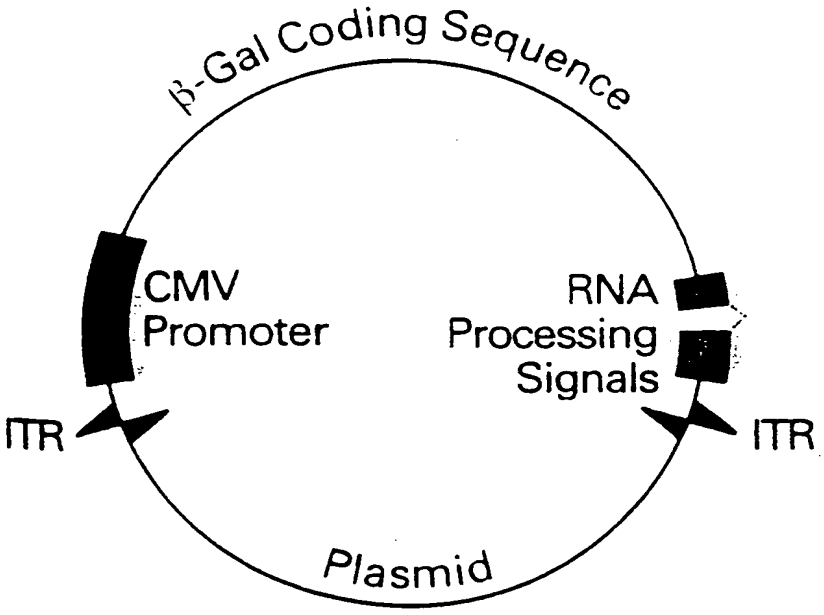

β-Gal Coding Sequence
CMV
Promoter
RNA
Processing
Signals
ITR
ITR
Plasmid

FIG. 10

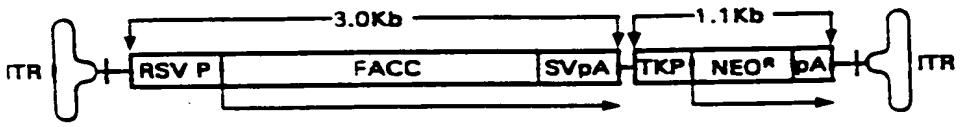


FIG. 11

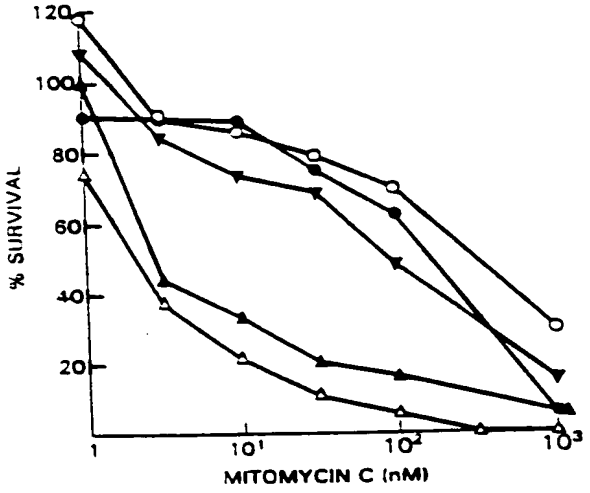


FIG. 12

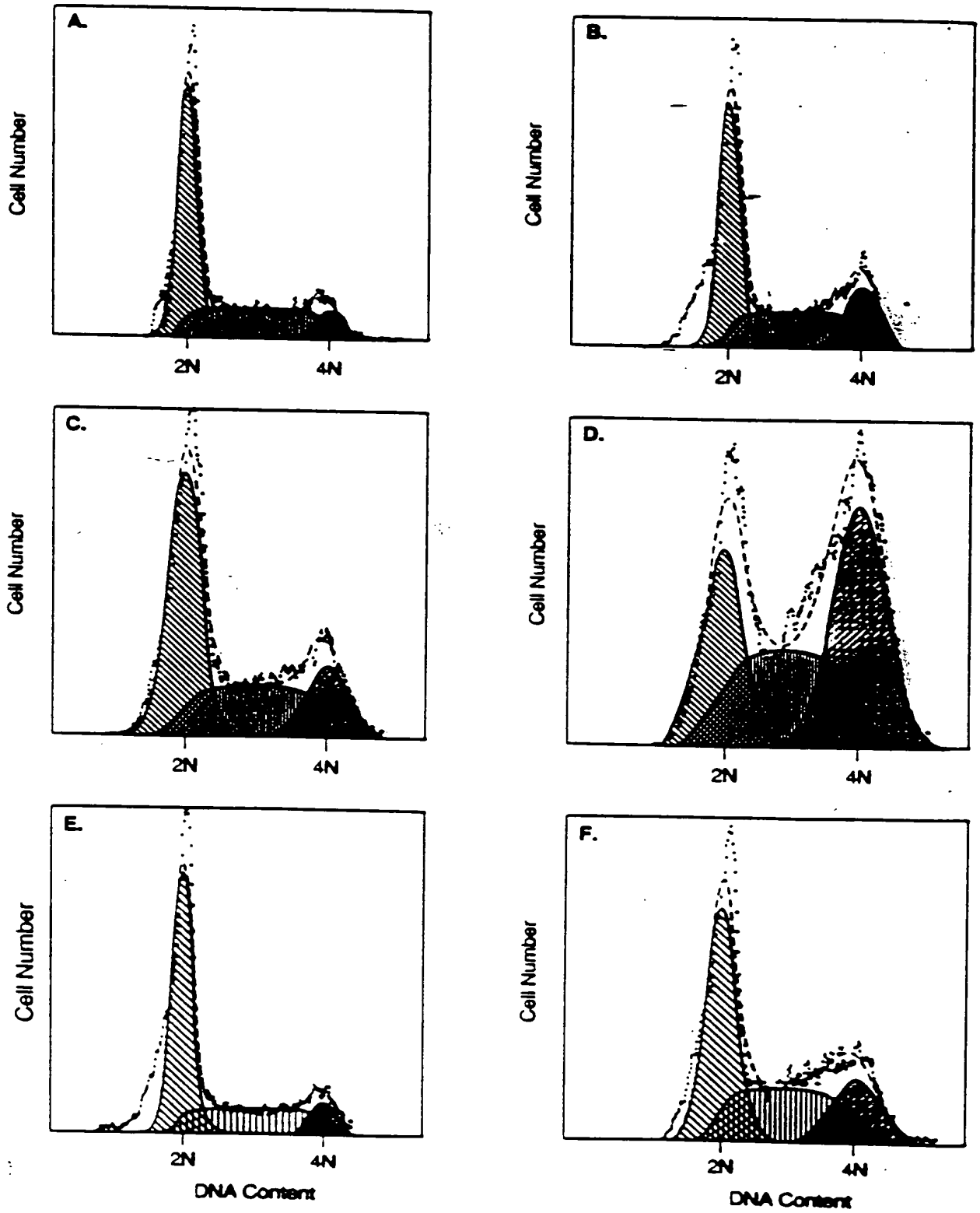
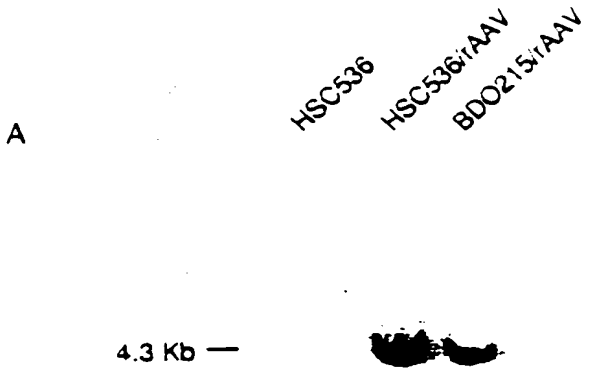


FIG. 13


A
HSC536
HSC536/rAAV
BDO215/rAAV
4.3 Kb

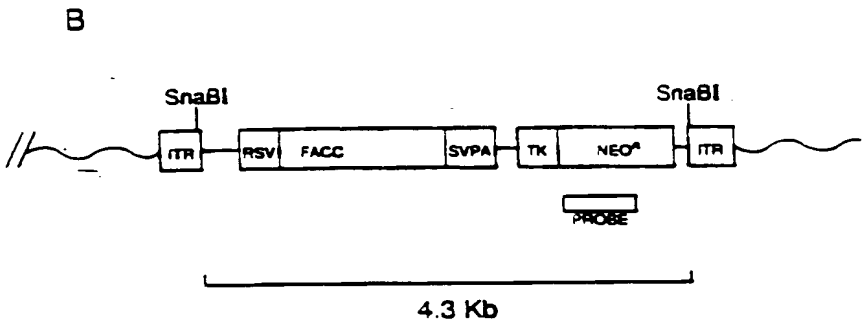
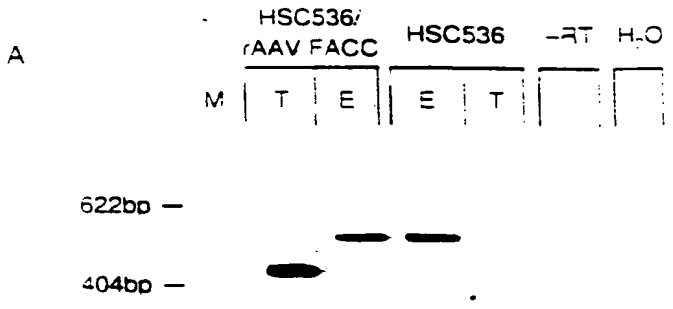

B
SnaBI
SnaBI
ITR
RSV
FACC
SVPA
TK
NEO
ITR
PROBE
4.3 Kb

FIG. 14


A
HSC536/
rAAV FACC
HSC536
-RT
H2O
M
T
E
E
T
622bp
404bp

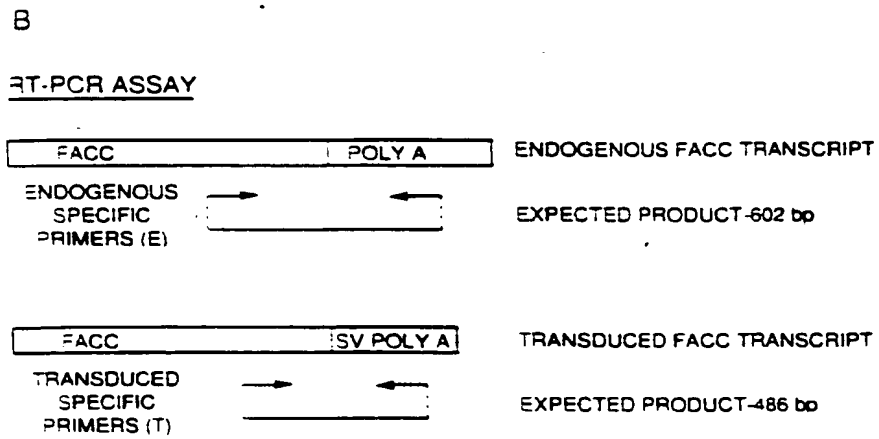

B
RT-PCR ASSAY
FACC
POLY A
ENDOGENOUS FACC TRANSCRIPT
ENDOGENOUS SPECIFIC PRIMERS (E)
EXPECTED PRODUCT-602 bp
FACC
SV POLY A
TRANSDUCED FACC TRANSCRIPT
TRANSDUCED SPECIFIC PRIMERS (T)
EXPECTED PRODUCT-486 bp

FIG. 15

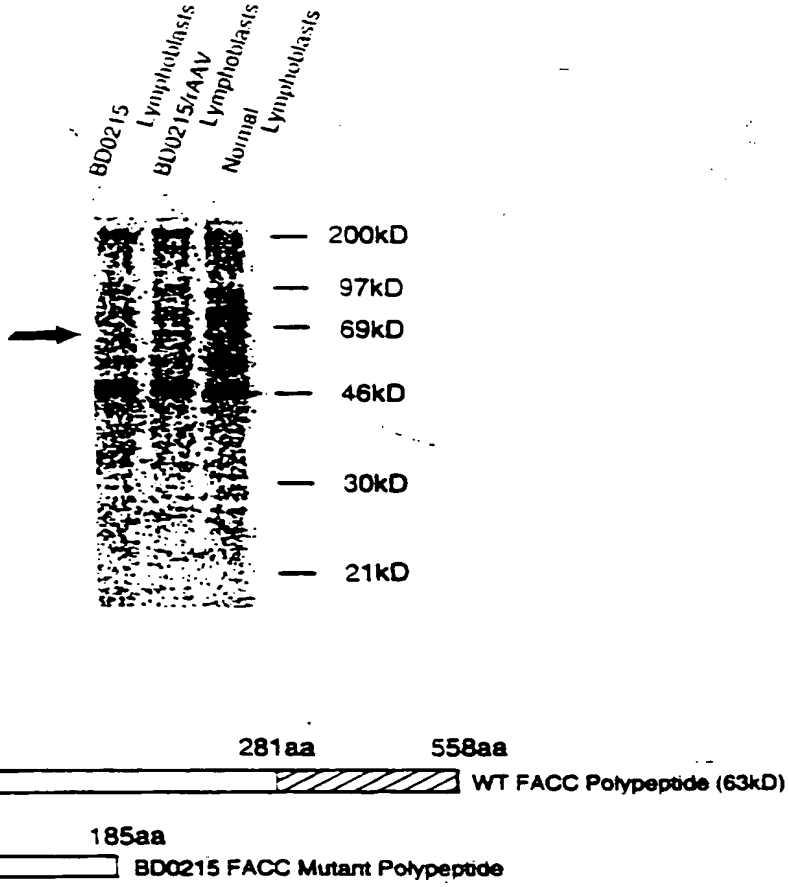

BD0215
Lymphoblasts
BD0215/rAAV
Lymphoblasts
Normal
Lymphoblasts
200kD
97kD
69kD
46kD
30kD
21kD
281aa
558aa
WT FACC Polypeptide (63kD)
185aa
BD0215 FACC Mutant Polypeptide

FIG. 16

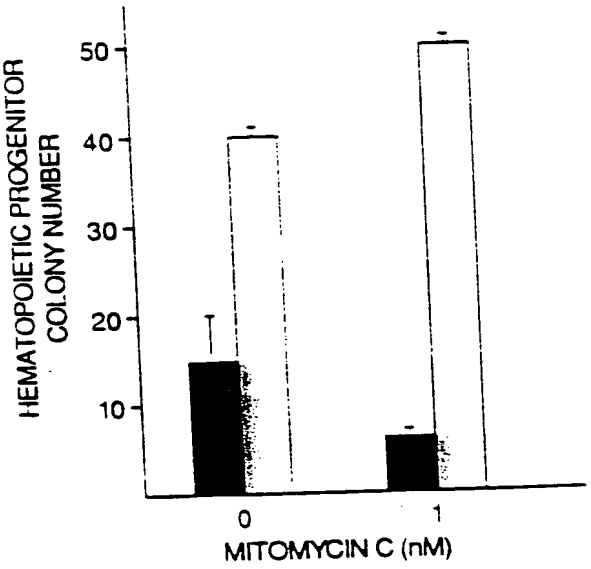

HEMATOPOIETIC PROGENITOR
COLONY NUMBER
50
40
30
20
10
0
1
MITOMYCIN C (nM)

FIG. 17

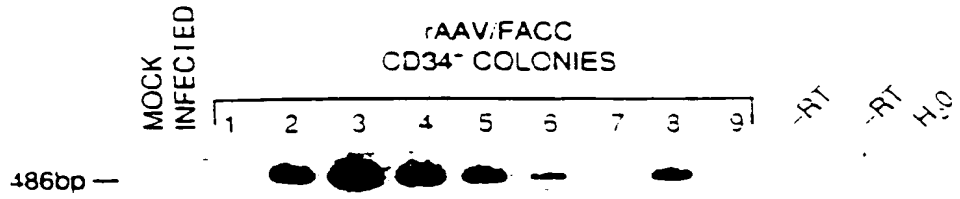

MOCK INFECIED
rAAV/FACC
CD34⁻ COLONIES
1
2
3
4
5
6
7
8
9
-RT
-RT
H2O
486bp

FIG. 18

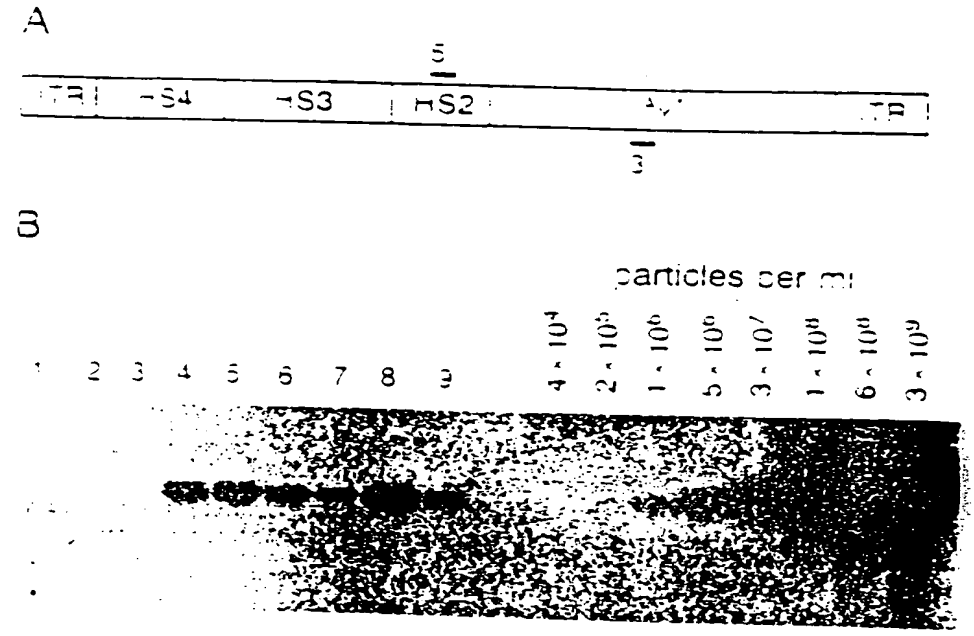

A
5
HS4
HS3
HS2
3
B
particles per ml
1 2 3 4 5 6 7 8 9
4 × 10⁴
2 × 10⁵
1 × 10⁶
5 × 10⁶
3 × 10⁷
1 × 10⁸
6 × 10⁸
3 × 10⁹
C
d
m

FIG. 19

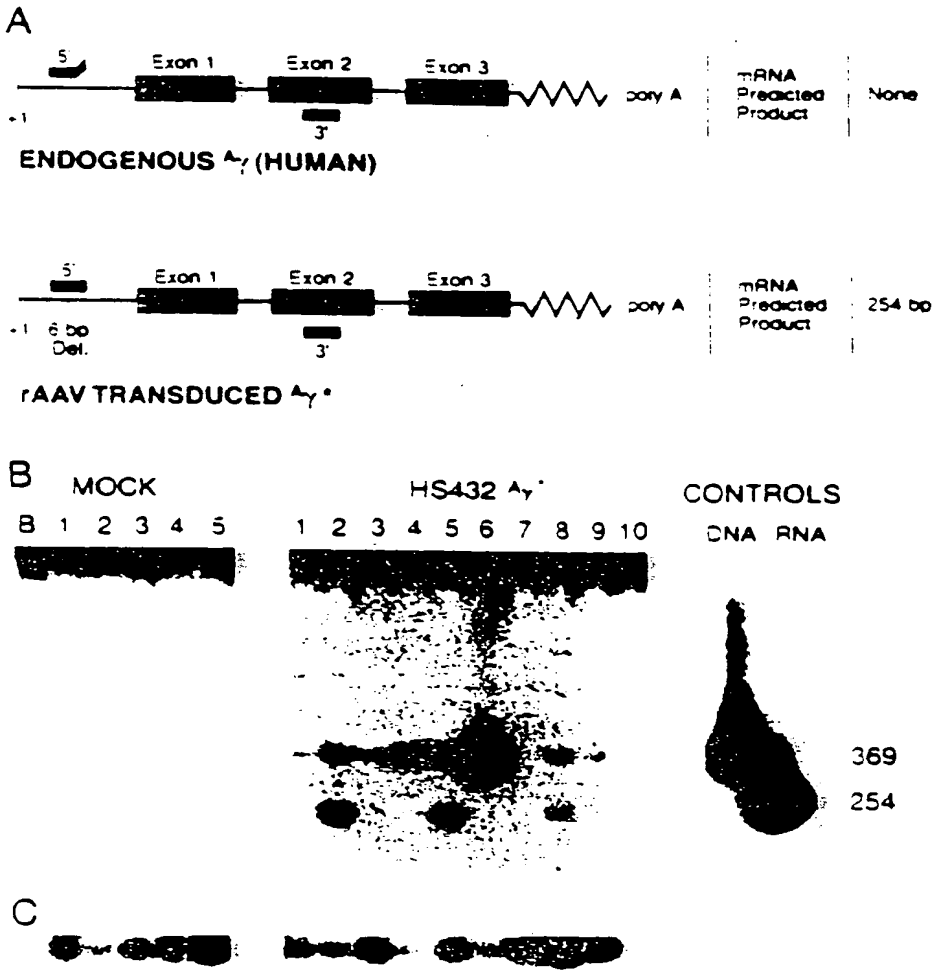

A
5'
Exon 1
Exon 2
Exon 3
poly A
+1
3'
mRNA Predicted Product
None
ENDOGENOUS Aγ (HUMAN)
5'
Exon 1
Exon 2
Exon 3
poly A
+1
6 bp Del.
3'
mRNA Predicted Product
254 bp
rAAV TRANSDUCED Aγ*
B
MOCK
B 1 2 3 4 5
HS432 Aγ*
1 2 3 4 5 6 7 8 9 10
CONTROLS
DNA RNA
369
254
C

FIG. 20

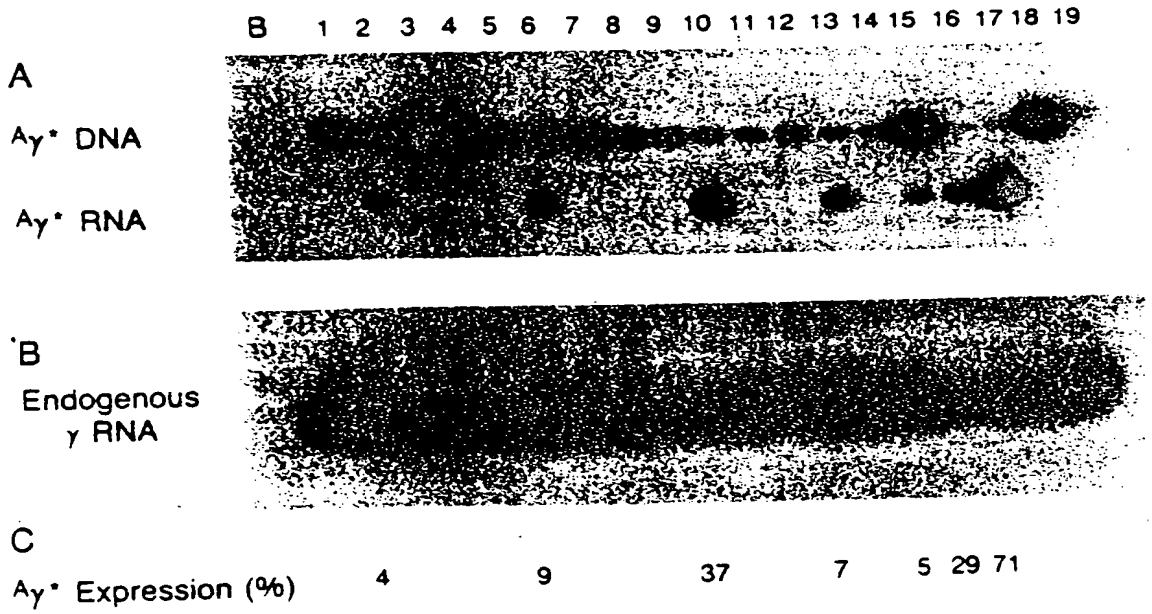

B 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
A
Aγ* DNA
Aγ* RNA
B
Endogenous γ RNA
C
Aγ* Expression (%)
4
9
37
7
5 29 71

FIG. 21

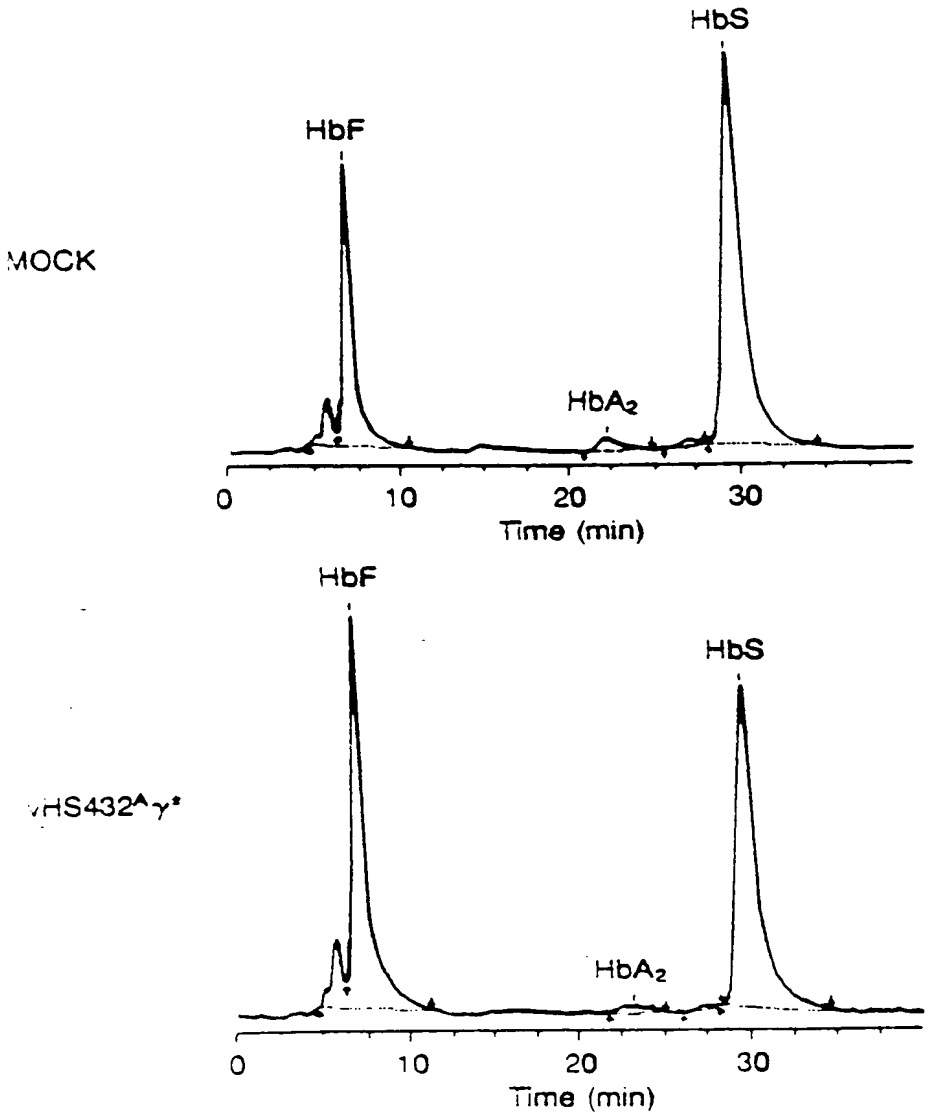

MOCK
HbF
HbS
HbA2
0
10
20
30
Time (min)
HbF
HbS
HbA2
0
10
20
30
Time (min)

Figure 1A

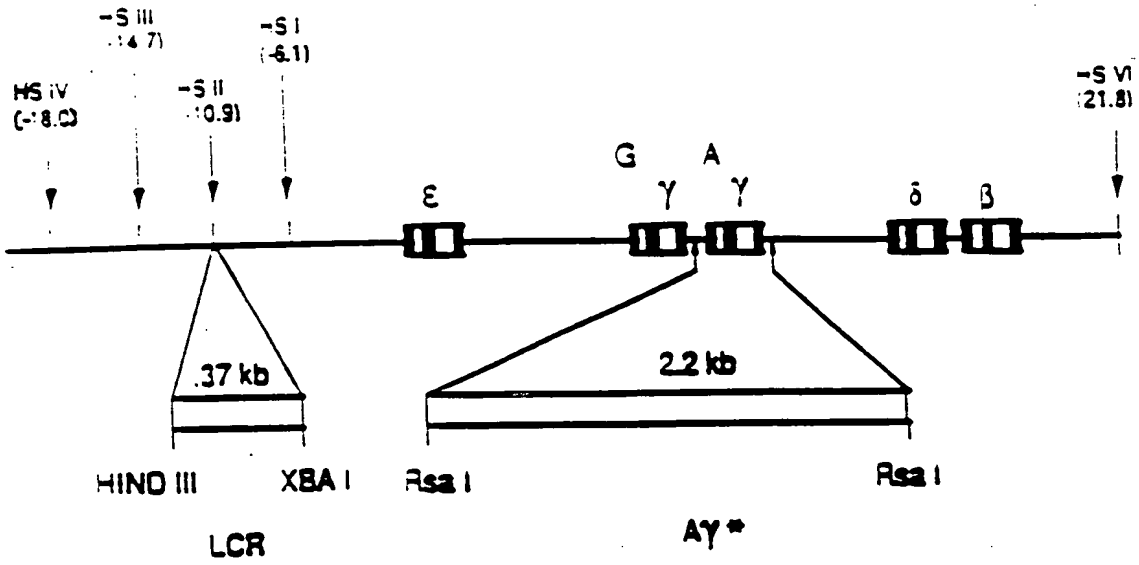


Figure 1B

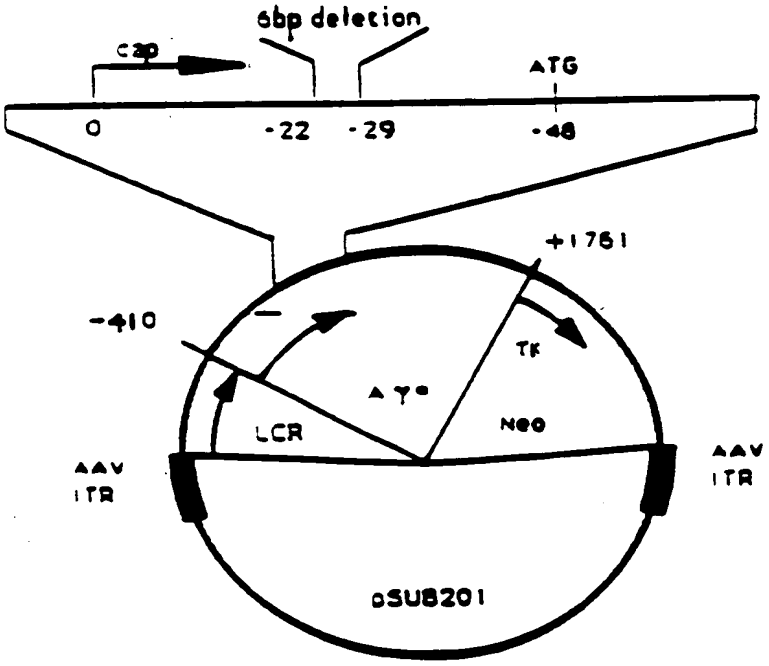


Figure 2A (+HS2) POOLS

Figure 2B (+HS2) CLONES

Figure 2C (-HS2)

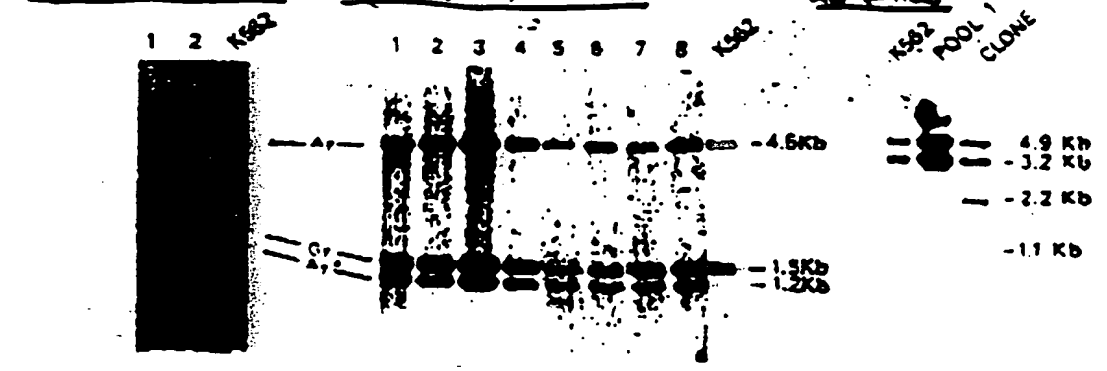


FIG 2D

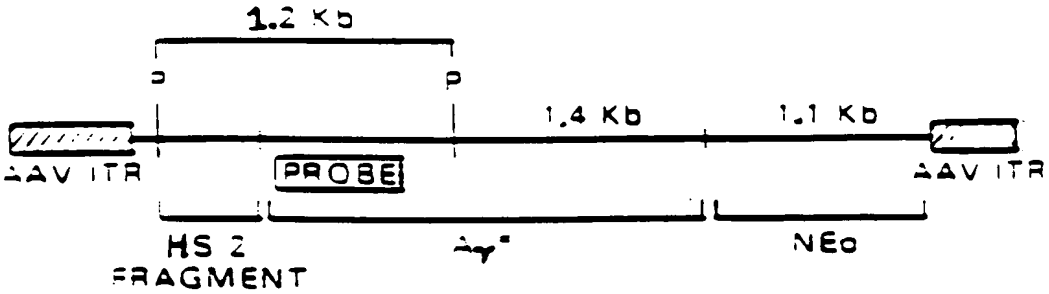


FIG. 3

RNase PROTECTION ANALYSIS OF rAAV/K562 POOLED CLONES

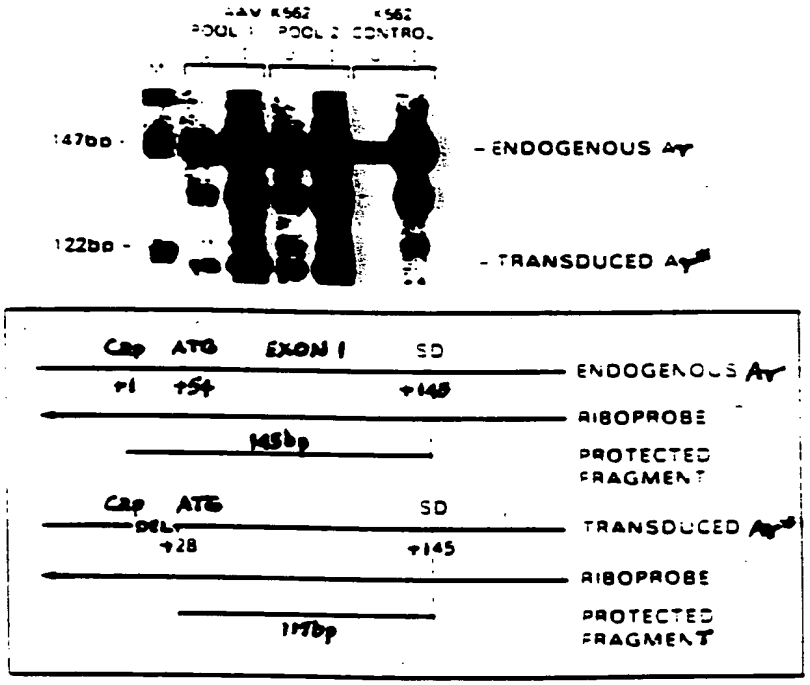


FIG. 4

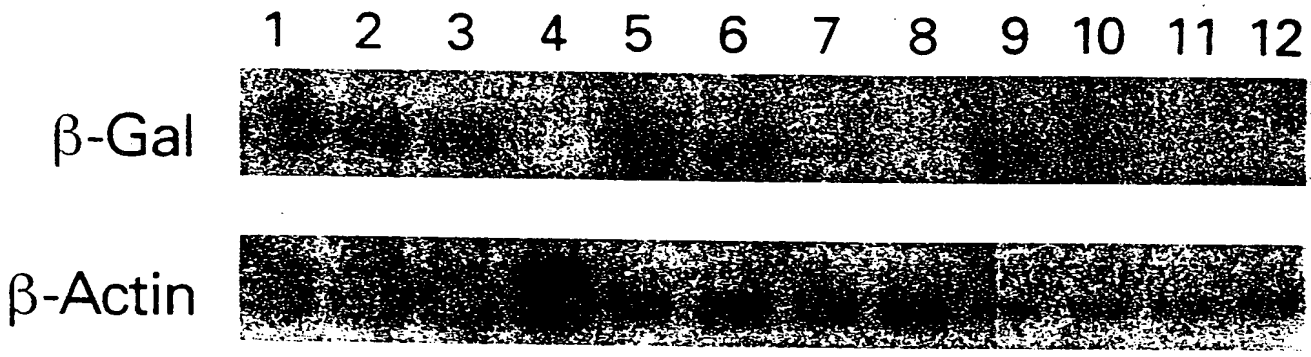


FIG. 5

FIG. 6

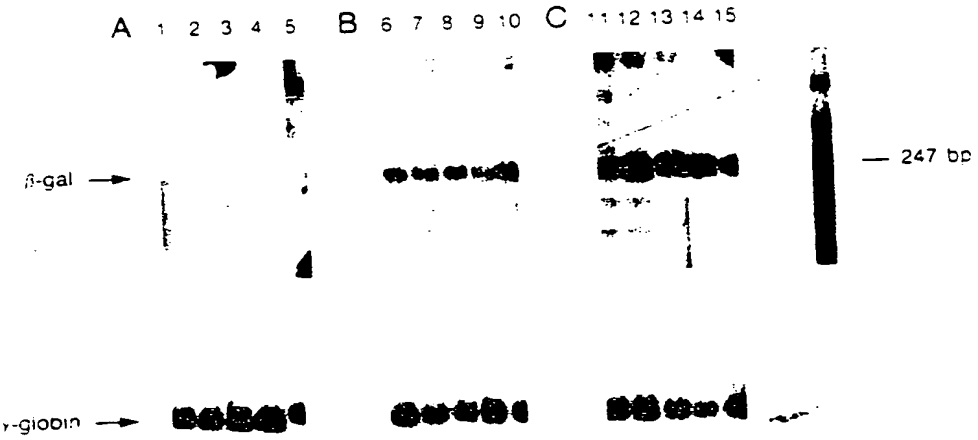


FIG. 7

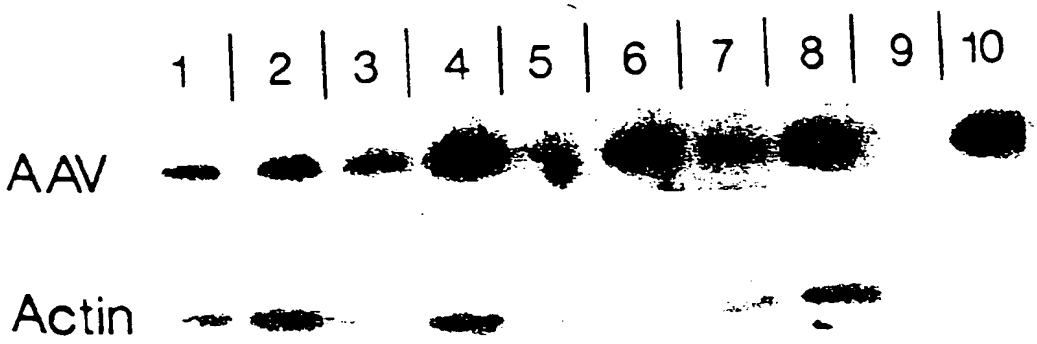
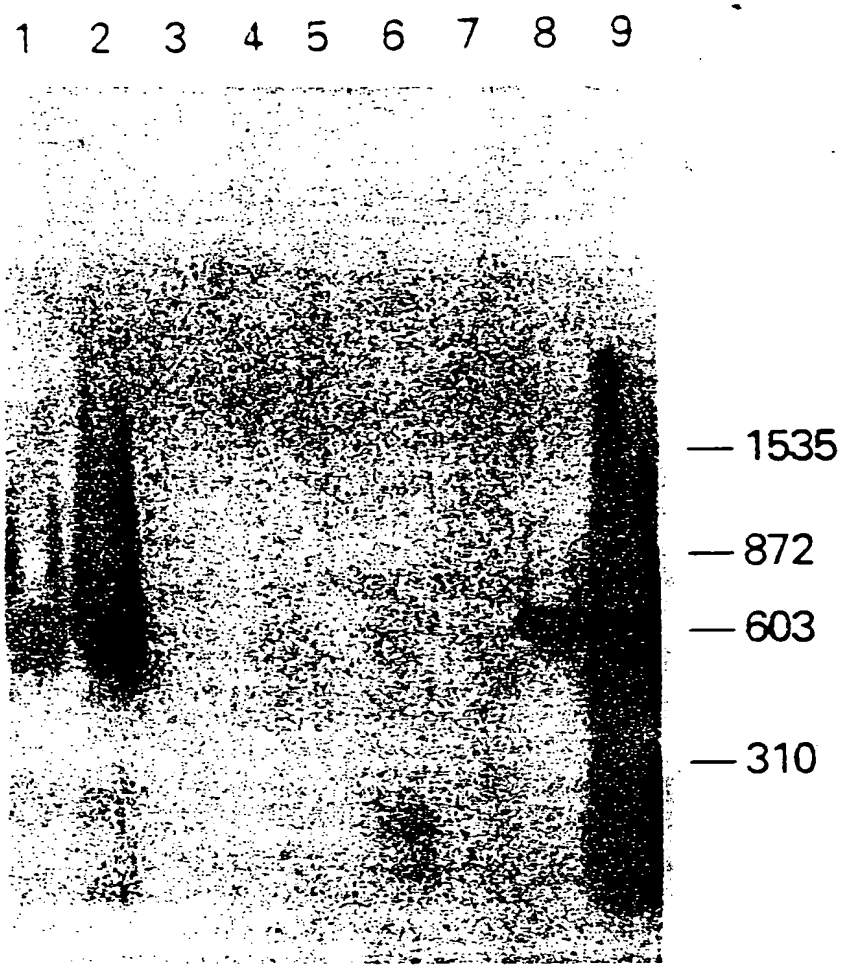


FIG. 8


1 2 3 4 5 6 7 8 9
1535
872
603
310

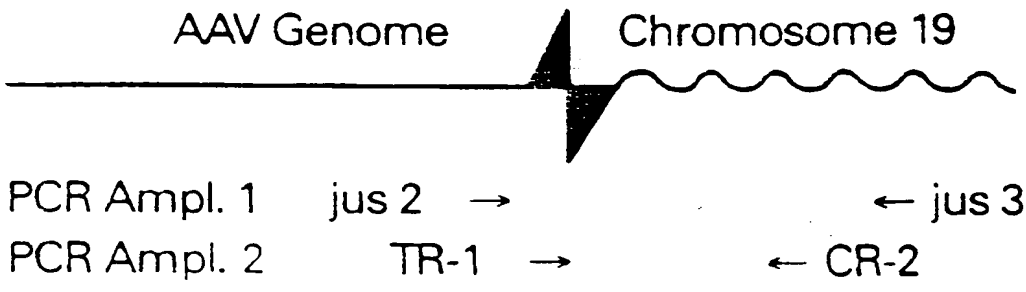

AAV Genome
Chromosome 19
PCR Ampl. 1
jus 2 →
← jus 3
PCR Ampl. 2
TR-1 →
← CR-2

FIG. 9

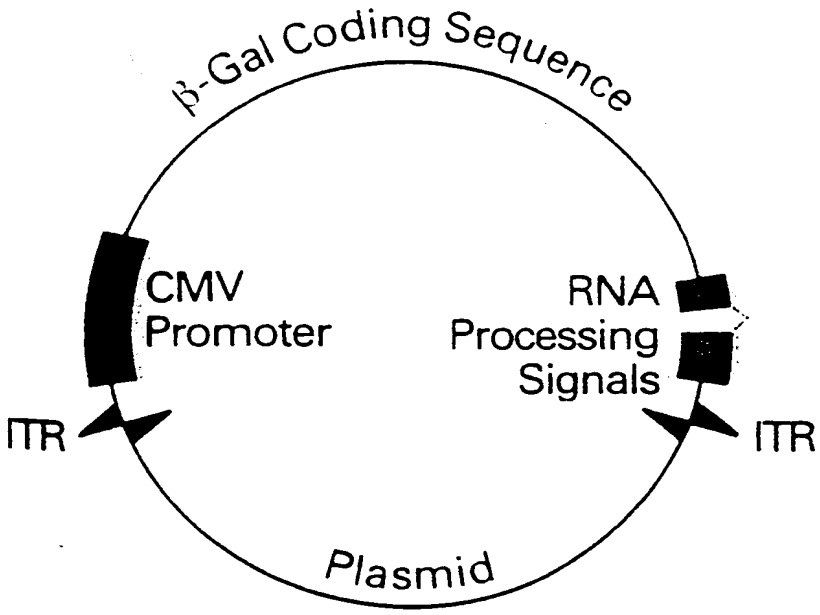

β-Gal Coding Sequence
CMV
Promoter
RNA
Processing
Signals
ITR
ITR
Plasmid

FIG. 10

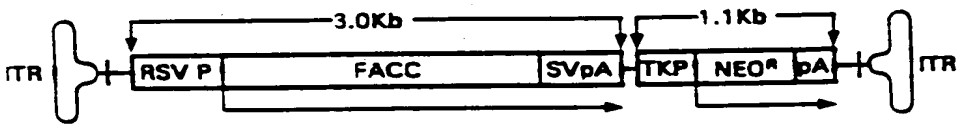


FIG. 11

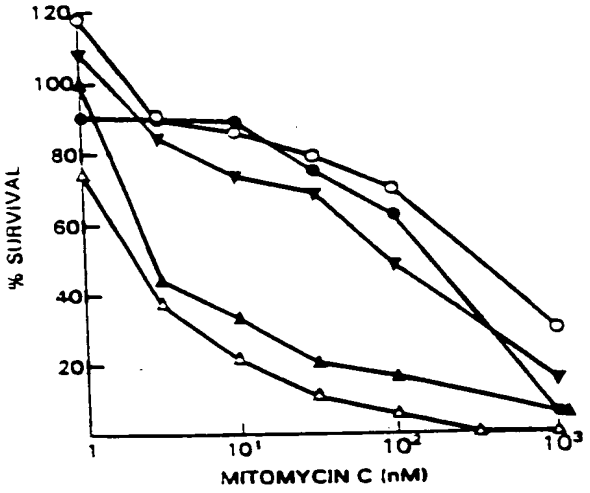


FIG. 12

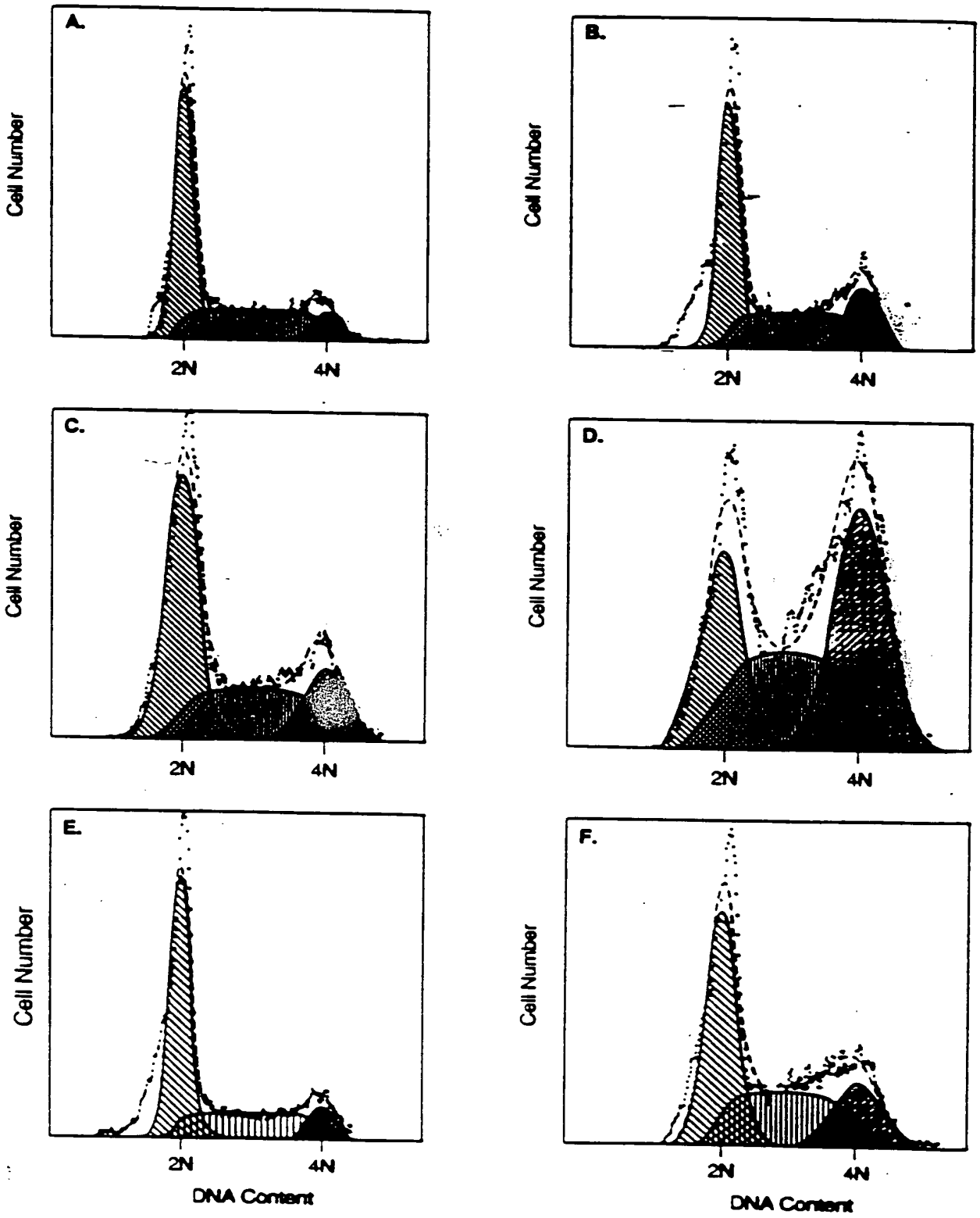
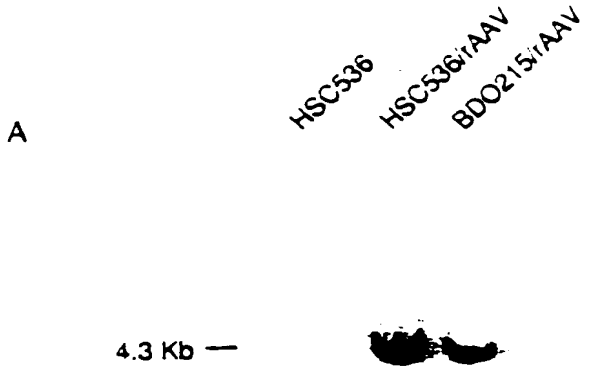


FIG. 13


A
HSC536
HSC536/rAAV
BDO215/rAAV
4.3 Kb

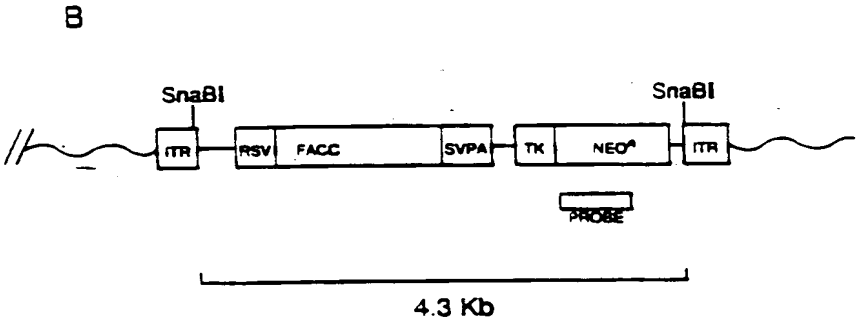
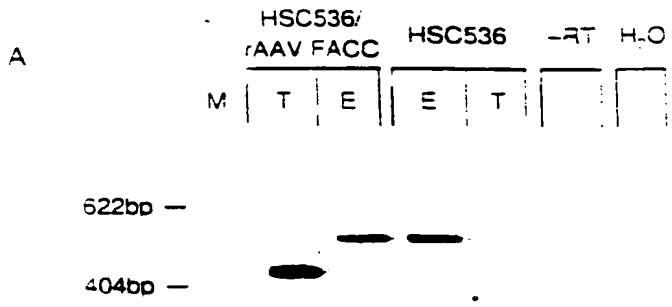

B
SnaBI
SnaBI
ITR
RSV
FACC
SVPA
TK
NEO
ITR
PROBE
4.3 Kb

FIG. 14



B

RT-PCR ASSAY

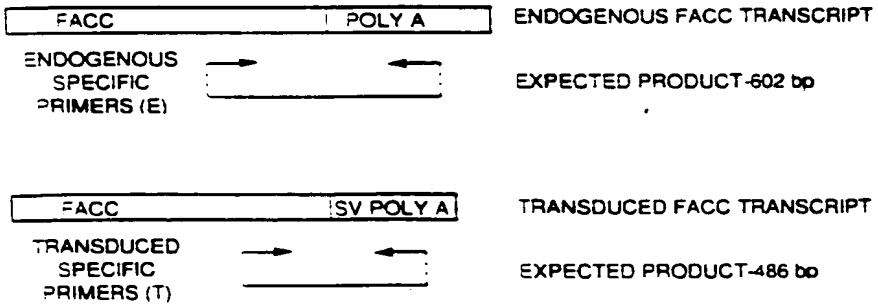


FIG. 15

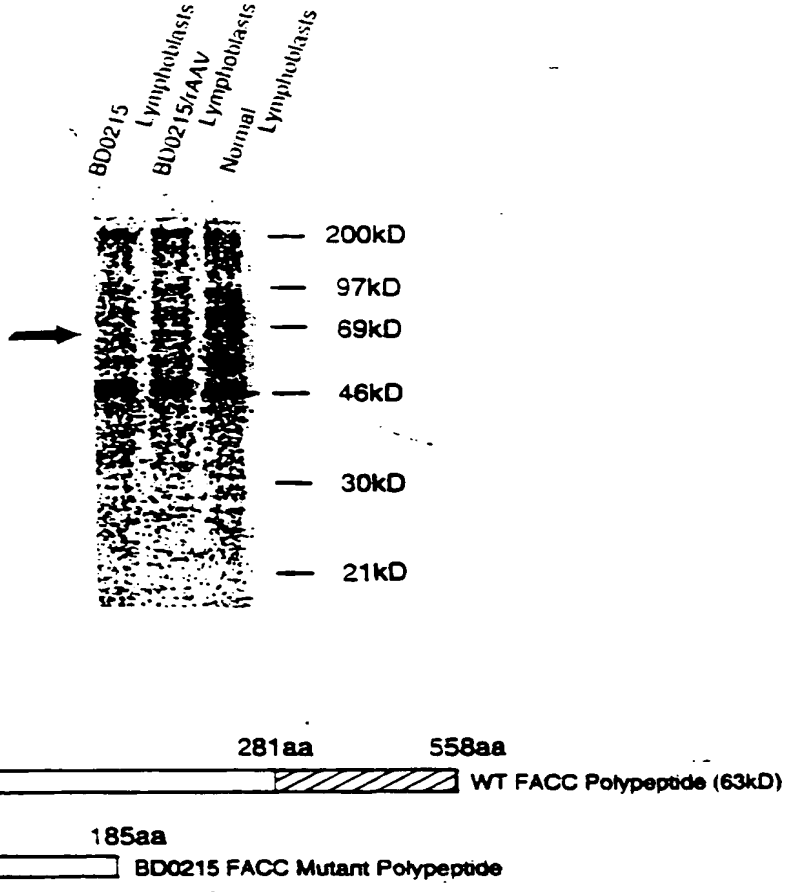

BD0215 Lymphoblasts
BD0215/rAAV Lymphoblasts
Normal Lymphoblasts
200kD
97kD
69kD
46kD
30kD
21kD
281aa
558aa
WT FACC Polypeptide (63kD)
185aa
BD0215 FACC Mutant Polypeptide

FIG. 16

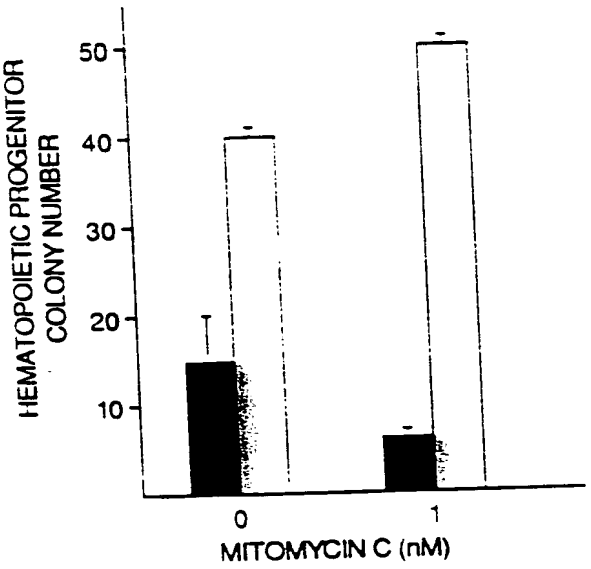

HEMATOPOIETIC PROGENITOR
COLONY NUMBER
50
40
30
20
10
0
1
MITOMYCIN C (nM)

FIG. 17

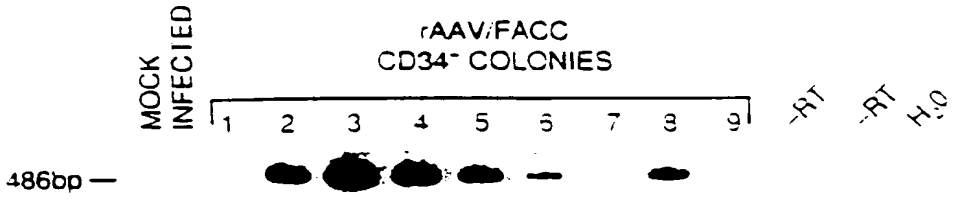

MOCK INFECIED
rAAV/FACC
CD34⁻ COLONIES
1 2 3 4 5 6 7 8 9
-RT
-RT
H2O
486bp

FIG. 18

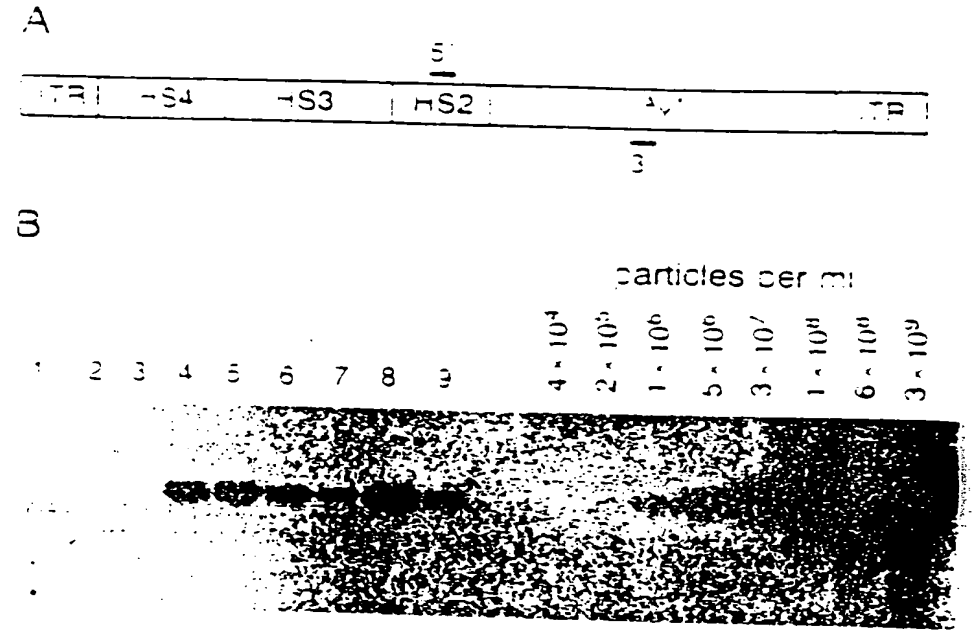

A
5'
TR
HS4
HS3
HS2
TR
3'
B
particles per ml
1 2 3 4 5 6 7 8 9
4 × 10⁴
2 × 10⁵
1 × 10⁶
5 × 10⁶
3 × 10⁷
1 × 10⁸
6 × 10⁸
3 × 10⁹
C
d
m

FIG. 19

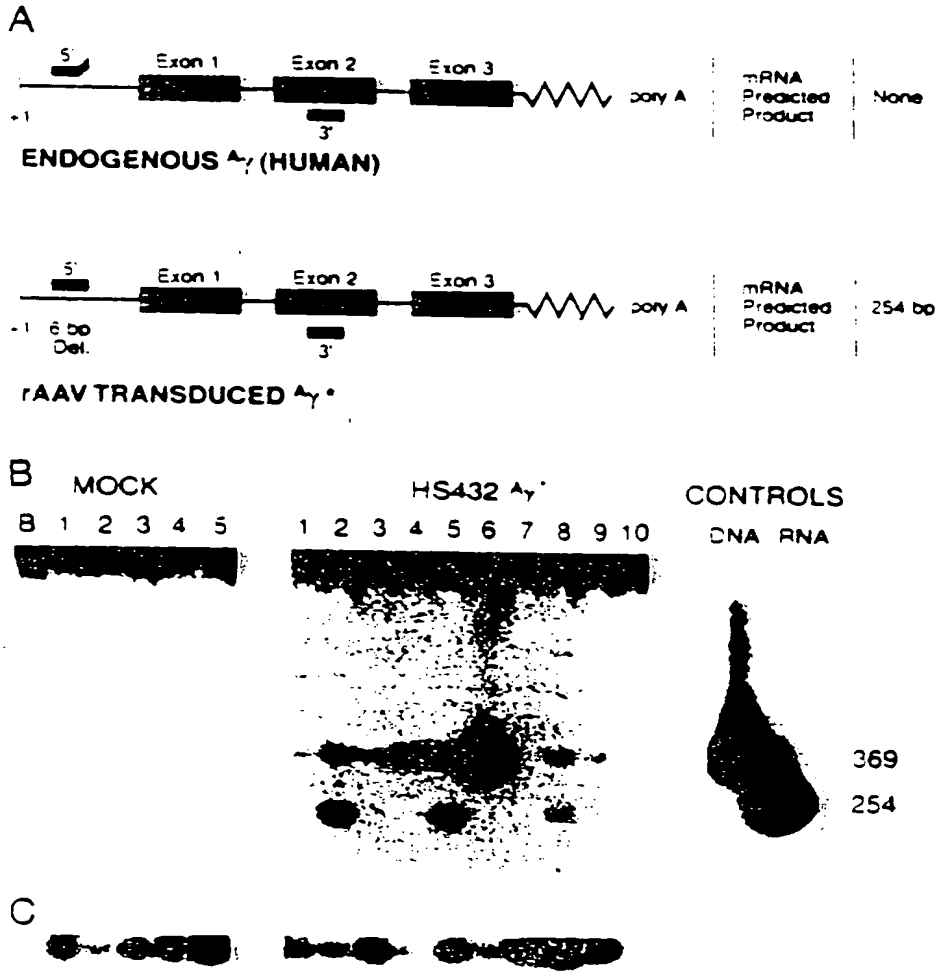

A
5'
Exon 1
Exon 2
Exon 3
poly A
+1
3'
mRNA Predicted Product
None
ENDOGENOUS Aγ (HUMAN)
5'
Exon 1
Exon 2
Exon 3
poly A
-1 6 bp Del.
3'
mRNA Predicted Product
254 bp
rAAV TRANSDUCED Aγ*
B
MOCK
B 1 2 3 4 5
HS432 Aγ*
1 2 3 4 5 6 7 8 9 10
CONTROLS
DNA RNA
369
254
C

FIG. 20

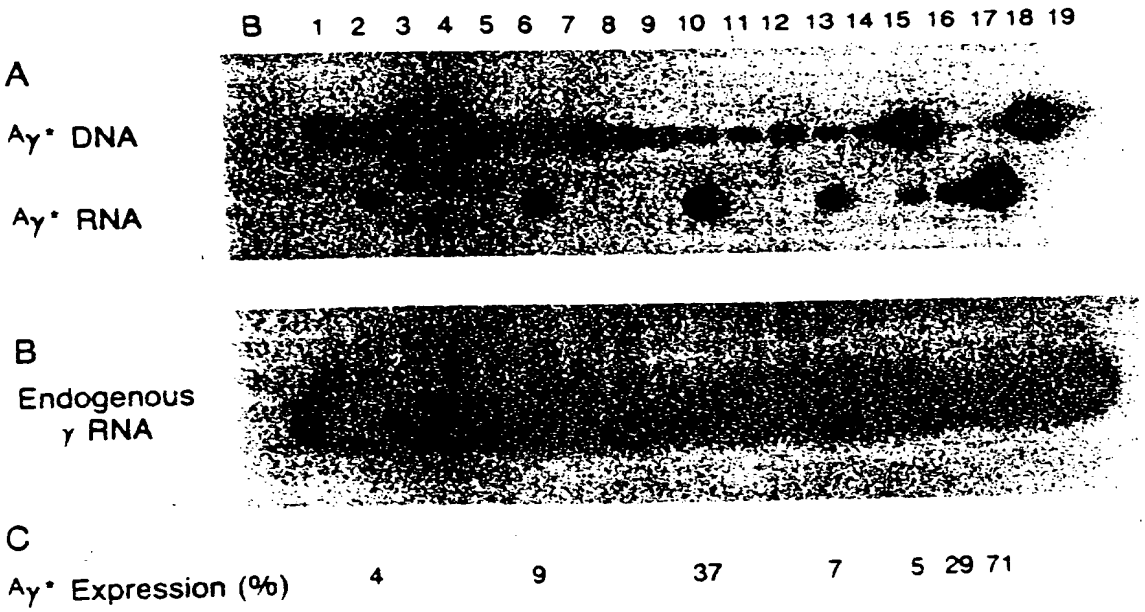
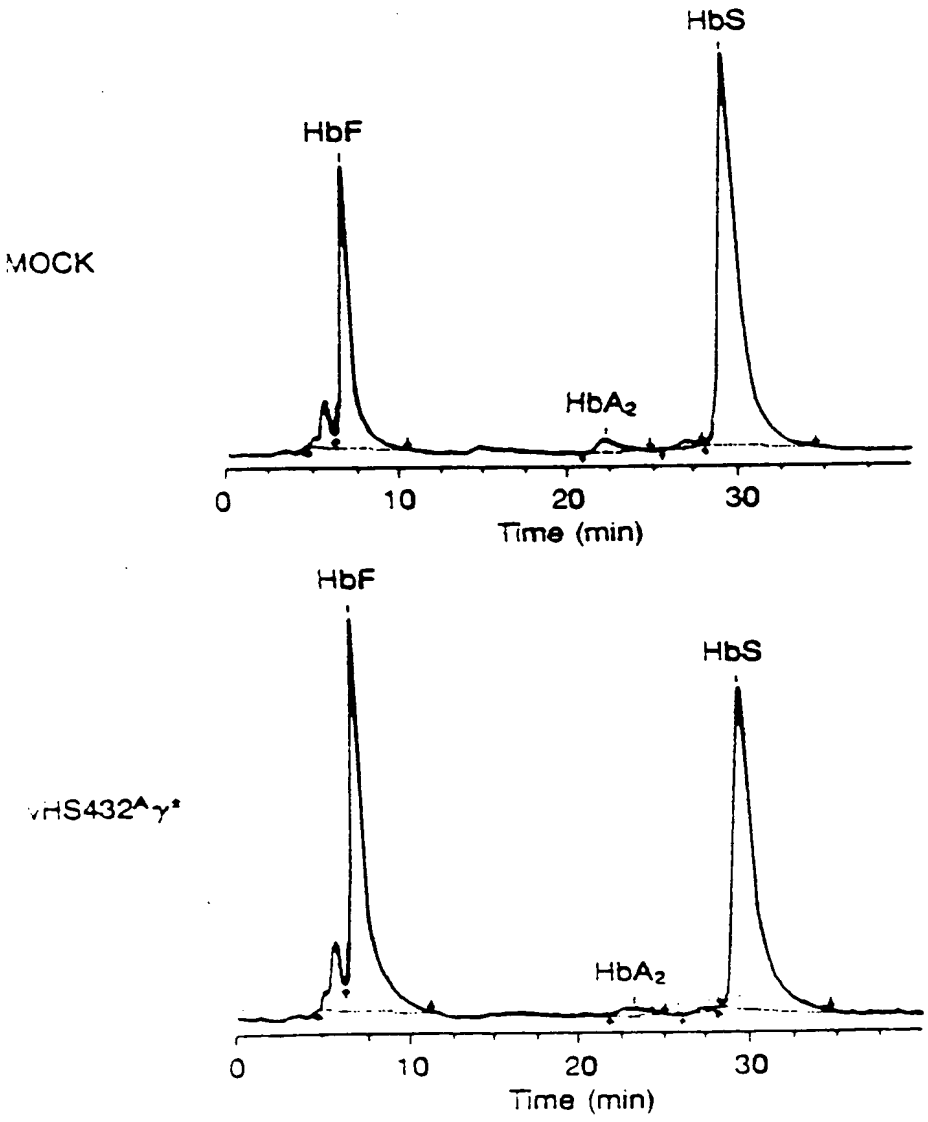

B 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
A
Aγ* DNA
Aγ* RNA
B
Endogenous γ RNA
C
Aγ* Expression (%)
4
9
37
7
5
29
71

FIG. 21


MOCK
HbF
HbS
HbA2
0
10
20
30
Time (min)
γHS432A
HbF
HbS
HbA2
0
10
20
30
Time (min)